PROCEEDINGS

SEVENTY-THIRD
ANNUAL MEETING

of the
UNITED STATES
ANIMAL HEALTH
ASSOCIATION

Sheraton - Schroeder Hotel
Milwaukee, Wisconsin
October 12, 13, 14, 15, 16, 17, 1969
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First Vice President

FRANK B. WHEELER  
President

W. C. TOBIN  
Second Vice President

W. L. BENDIX  
Secretary-Treasurer
## RECORD OF PREVIOUS MEETINGS

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<th>Secretary</th>
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<td>26. Dec. 6-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
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<tr>
<td>27. Dec. 5-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Mont.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
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<td>Date</td>
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<td>Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>Dr. Peter Malcolm</td>
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<td>Dr. E. T. Faulder</td>
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<tr>
<td>Sept. 6-8, 1934</td>
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<td>Dr. T. E. Robinson</td>
<td>Providence, R.I.</td>
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<td>Dec. 4-6, 1935</td>
<td>Chicago, Ill.</td>
<td>Dr. Edward Records</td>
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<td>Dec. 24, 1936</td>
<td>Chicago, Ill.</td>
<td>Dr. Walter Wisnicky</td>
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<td>Dr. R. W. Smith</td>
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<td>Dr. D.E. Westmoreland</td>
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<td>Dr. J. L. Axy</td>
<td>Indianapolis, Ind.</td>
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<td>Dec. 4-6, 1940</td>
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<td>Dr. H. D. Port</td>
<td>Cheyenne, Wyo.</td>
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<td>Dr. I. S. McAdory</td>
<td>Auburn, Ala.</td>
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<tr>
<td>Dec. 1-3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks</td>
<td>Salt Lake City, Utah</td>
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<tr>
<td>Dec. 6-8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton</td>
<td>Atlanta, Ga.</td>
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<td>Dec. 5-7, 1945</td>
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<td>Dr. C. U. Duckworth</td>
<td>Sacramento, Calif.</td>
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<td>Chicago, Ill.</td>
<td>Dr. William Moore</td>
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<td>Chicago, Ill.</td>
<td>Mr. Will J. Miller</td>
<td>Topeka, Kan.</td>
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<td>Denver, Colo.</td>
<td>Dr. Jean V. Knapp</td>
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<tr>
<td>Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>Dr. T. O. Brandenburg</td>
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<tr>
<td>Nov. 14-16, 1951</td>
<td>Kansas City, Kan.</td>
<td>Mr. F. E. Mollin</td>
<td>Denver, Colo.</td>
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<tr>
<td>Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>Dr. T. Childs</td>
<td>Ottawa, Canada</td>
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<tr>
<td>Nov. 10-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green</td>
<td>Charleston, W.Va.</td>
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<tr>
<td>Nov. 16-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins</td>
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<tr>
<td>Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner</td>
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<td>Nov. 13-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good</td>
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<td>Date</td>
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<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, Fla</td>
<td>Dr. John G. Milligan, Montgomery, Ala.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<td>63. Dec. 15-18, 1959</td>
<td>San Francisco, Calif.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
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<td>65. Oct. 3-Nov. 1-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, N.M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R.I.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<td>70. Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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*Deceased  †Reprinted in 54th Annual Report  ††Reprinted in the 66th Annual Report
+This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION

HARRY E. GOLDSSTEIN, D.V.M.

Almighty God, our Heavenly Father, we give Thee thanks that we are again privileged to meet and discuss our various problems and report upon the progress gained this past year.

We beseech Thee to bless this association which has now changed its name to the United States Animal Health Association. We wish to thank Thee for a fruitful year and implore Thy Guidance and Blessing for our future endeavors.

Grant that the activities of this our Seventy-third Convention will be found pleasing to Thee.

We desire to commence, to continue and to conclude our discussions and deliberations in the remembrance of Thine own existence.

Direct us in all our doings that we may glorify Thy Holy name——

Amen.
MEMORIAL SERVICE

Harry E. Goldstein, D.V.M.
Columbus, Ohio

President O'Hara, Distinguished Guests, Ladies and Gentleman —

We are assembled in this session to pay tribute to the memory of those members of this association who have endeared themselves to this association, and have passed on since our last meeting.

We, the living, sometimes fail to properly evaluate the works that come before us. In an era where man can visit the moon, and reach even further in the universe, we lose sight of what is near and dear to us — our fellowship.

There are no words, phrases or paragraphs that can adequately convey properly the true sentiments of recognition for those who are no longer on our roster.

Their deeds, feats and contributions speak for themselves.

So that the memory of our departed members may become recorded, let the record show:

STANLEY J. BOWER — (Michigan State University 1955) — died October 31, 1968
SAMUEL D. BUZZARD — (Cincinnati Veterinary College 1915) — died November 27, 1968
JOHN R. DAY — (Kansas City University 1917) — died October 4, 1968
THOMAS A. GAGE — (Cornell University 1943) — died September 11, 1968
HARRY L. FOUST — (Ohio State University 1914) — died August 15, 1968
HARRY E. KEMPER — (Kansas City Veterinary College 1914) — died December 7, 1968
R. R. PHARR — (Kansas City University 1917) — died June 15, 1968
WILLIAM M. THOMPSON — (Cornell University 1916) — died July 3, 1968
C. PAUL VICKERS — (University of Auburn 1937) — died December 30, 1969
E. P. ALTHOUSE — (University of Pennsylvania 1903) — died May 12, 1969
FRANKLIN SUITS — (Kansas City University 1916) — died May 11, 1969
HARRISON N. WAITE — (Cincinnati Veterinary College 1915) — died May 23, 1969
RUBEN BLACKNER — (Kansas City University 1918) — died July 3, 1969
EDWARD R. CUSHING — (Cornell University 1920) — died July 2, 1969
ALDIE P. INNENSCUH — (Kansas City University 1941) — died July 2, 1969
CHARLES C. DURBIN — (University of Pennsylvania 1949) — died January 5, 1969
HERMON P. HONSTEAD — (Kansas State University 1950) — died April 15, 1969
FLEETWOOD R. KOUTZ — (Washington State University 1936) — died April 6, 1969
HARRY W. SCHOEMING — (University of Pennsylvania 1907) — died April 12, 1969

May be bow our heads for a moment of silent prayer and tribute. Amen.

xx
MESSAGE OF THE PRESIDENT-ELECT

by

Frank B. Wheeler, D.V.M.

United States Animal Health Association

At the annual meeting in 1909, this organization changed its name from "The Association of Interstate Livestock Sanitary Boards" to "The United States Livestock Sanitary Association."

It so happened that this was done under the Presidency of Dr. W. H. Dalrymple, a very distinguished scholar, educator, and veterinarian from Louisiana. Now, 60 year later, it appears history is repeating itself. The organization has again changed its name and, coincidentally here stands another Louisiana veterinarian, however, one without distinction or learned accomplishments, making the President-Elect's address.

The world has changed dramatically since the horse and buggy days of Dr. Dalrymple and so has the field of regulatory veterinary medicine. The problems today are more numerous and much more complicated. We are told to expect just as much change during the next 10 years as has occurred in the last 100. If this is an accurate prediction, we have no time to waste and should be preparing to meet the challenge. We must be prepared to change in areas when change is indicated in order to overcome weakness and to develop strength for the future.

Yes, changing the name of our organization is important and is certainly in order, but unless we can meet the needs of today and the challenge of tomorrow our name plate has little significance.

The 1970's will present the United States Animal Health Association with its greatest challenge but, at the same time, will give our organization its greatest opportunity to serve the livestock industry. There is no question that foreign animal diseases will gain entrance into the United States, either by accident or design. All segments of the livestock industry should be prepared physically, psychologically and economically to cope with the ordeal. The enlightenment and involvement of the industry should be going on NOW during the calm before the storm rather than in its midst when there is no time to explain nor a desire to listen. Failure to prepare ourselves and the industry for such an eventuality would be painfully embarrassing to State-Federal Regulatory Officials and a staggering setback to the industry we are pledged to protect.

In the future, animal health officials will no longer be able to ignore coming to grips with potential disease problems until after the disease has become established and the treat a reality. This is the way it was done in the past — the easy way. It turned out to be, however, the most expensive, least professional way and certainly did not exemplify leadership on the part of animal health officials.

Presently we are guilty of this same negligence as there is an urgent need to develop eradication programs for diseases that are already well established and spreading yet are virtually unchallenged. Anaplasmosis is a prime example. The tools of eradication have been available for sometime but no significant effort has been made to put them to use. Meanwhile, the principal reservoir of infection — the
carrier animal — is increasing in number in logarithmic progression year by year. In the future, we must have the intelligence to recognize the potential threat of a disease and then have the ability and determination to promote its immediate elimination.

The greatest opportunity for the United States Animal Health Association to serve will be in providing leadership to complete the transition from the old approach to disease control to an approach based on early detection and quick elimination, thereby preventing disease from developing into serious threats. Making this change to preventive animal health programs will demand an improved system of disease reporting; the development of continual comprehensive screening procedures of all livestock herds; accurate diagnostic screening tests to detect foreign and domestic diseases most likely to threaten the livestock industry; practical and efficient track-back procedures to the herds of origin, and cooperation of State and Federal meat inspection personnel.

Possibly the most difficult aspect of such a transition will be selling the livestock industry on the economic and practical soundness of such a preventive approach. The individual livestock owners must be convinced that early disease detection and elimination is the most effective means of preventing major outbreaks of foreign and domestic disease. The endorsement and support of these proposed programs by livestock organizations will be essential.

Unless this transition to preventive regulatory veterinary medicine is completed in the near future, the delay could be disastrous to the livestock industry. Meat would then be beyond the economic means of the people. Soybeans would become the major protein source of the masses with beef only for the palates of the rich.

It would be tragic indeed, if not criminal, if we, the Livestock Disease Control Officials, being aware of this possibility do nothing to prevent it.

To be better prepared to meet the challenge of the 1970's we must increase our effectiveness. This can best be done by objectively examining ourselves and our relationship with each other and the livestock industry we serve. In this way we can recognize our weaknesses and correct them; know our strong points and further develop them.

Fully realizing that what I am about to say may not be well received and that I may be treading on "Hallo'ed Ground" and perhaps kicking someone's sacred cow, I would like to take advantage of this opportunity to mention several areas of deficiency which need our immediate attention. I am certainly not setting myself up as an efficiency expert but merely one who has been in the business for 22 years and has come to some definite conclusions. Please do not assume Louisiana has none of these deficiencies — I suspect I can match deficiencies with any State Veterinarian in the Association.

STATE-FEDERAL RELATIONS

Let me begin by kicking that "Fuzzy Federal Cur" around a bit. I should like to emphasize first of all, however, that the relationship between State and Federal Regulatory agencies is better today than at any time I can remember, even though there are some areas where improvement is still needed. The accomplishments of the joint cooperative efforts over the years far outweigh the failures. In other words, State-Federal relations could very appropriately be
compared to sexual relations – even when they’re bad, they’re good.

The recent Congressional action requiring compulsory meat inspection throughout the country will prove to be one of the most significant advancements made in recent years. It will not only protect the consumers but will be an important cog in the future mechanics of preventive animal disease programs. We may “gripe” about Federal encroachments on states’ rights but I ask you where would States be without the pressure of Federal Government to make us upgrade our programs from time to time? All of us know how difficult it is to put pressure on ourselves and how dependent we are on outside forces to make us face issues. There is no doubt the States need the Federal Government and vice versa, but it must be kept in proper balance.

The success or failure of National Animal Health Programs depends primarily on the ability of the State-Federal animal health agencies to cooperate. Nationally the Federal Government is, and should be, the dominant force in the implementation of national livestock disease control programs but in too many instances the Federal Animal Health Division dominates within the states as well. This domination is not necessarily the result of Federal design but rather the inability or unwillingness of the states to assume their obligations in this respect. This problem can be overcome when the states provide sufficient funds to enable their participation in State-Federal programs on an equal basis and thereby eliminate the need for the Federal Government to assume the greater burden of both funds and personnel. Certainly none of us are so naive as to believe the party who finances the largest portion of a joint venture will not dominate it. Unless this trend of letting Uncle Sam bear the burden is reversed, we will no longer have joint State-Federal programs – just Federal ones.

I believe it would be useful to define responsibility of the State and Federal Animal Disease Agencies within a state. This should not be difficult as it is commonly accepted the state has the sole authority and responsibility for the development and implementation of the livestock disease programs within the state except, of course, in the event of a National Emergency. The Federal Animal Health Agency, on the other hand, has a twofold role. First, to assist the State in the implementation of programs of national interest. The second and most neglected role is to represent the animal health interest of the other 49 states.

In the first category the Federal Government is in the State by invitation to assist the State, but in the second it is there by necessity, with or without an invitation. In the event the laws and regulations of a state are inadequate to support effective Animal Health Programs, or it fails to maintain minimum standards of national programs, these facts should be made known. Then if the state is unwilling or unable to correct the situation, the Federal Government, in conjunction with the other states, should use whatever pressure tactics at their disposal including, if necessary, interstate restrictions until the situation is corrected and the threat to the remaining states removed. This coordinated State-Federal action should prove much more effective than the present means by which states attempt to protect themselves by imposing local restrictions, or completely prohibiting livestock movements from the deficient states. Unilateral action hardly ever corrects the situation but it does further complicate an already complicated interstate movement problem.
LACK OF STANDARDIZATION OF STATE-FEDERAL PROGRAMS AND LACK OF UNIFORM ENFORCEMENT OF FEDERAL REQUIREMENTS

In spite of the fact State-Federal programs are supposed to be the same throughout the United States, there are alarming differences. There are instances where fundamental requirements are being ignored in some states and, even though it is known to Federal officials domiciled there, nothing is done to correct it. In spite of this the program status of the state remains unaffected. The neighboring state officials are usually aware of the deficiencies and that nothing is being done to correct them. This results in a loss of confidence in the State-Federal programs and a decline in the significance of the various "status" tags placed on counties and states. Once again, the surrounding states attempt to protect themselves by imposing restrictions on livestock originating from these areas which would otherwise be moving freely in interstate commerce, or at least with fewer restrictions. Surely something must be done to restore the confidence of the industry in the State-Federal eradication programs and to prevent this lack of standardization and uniformity in programs that are to be developed in the future.

I understand some state livestock disease control agencies have very little, if any, statutory authority upon which to build effective programs and must depend on a voluntary or optional basis. This means there are counties and perhaps states being recertified in the Brucellosis Eradication Program on the basis of market cattle tests when the backtagging of cattle is optional. Certainly this does not represent continual, cross-section sampling of all cattle herds. Recertification of counties or states in which the herds most likely to be infected with brucellosis are not being backtagged, and their owners are not required to have periodic herd tests, is a farce.

It is also discouraging to learn Federal requirements are not being enforced in all states. This makes it difficult to explain why certain requirements are placed on cattle in the Federally approved markets in one state but not in another. Apparently nothing effective is being done to assure uniform enforcement. It is my "flatfooted" opinion the failure to uniformly enforce a Federal regulation in all states should prohibit its enforcement in any state.

It would serve a very useful purpose if a comprehensive study were made to determine the basic Animal Health authority of each state, the compliance of each state in regard to established standards of the various State-Federal programs, and whether or not federal regulations are being uniformly enforced. Such a study would indicate where adjustments must be made in order to assure uniform programs and standards throughout the nation.

CONTINUING EDUCATION AND TRAINING

The continuing training of state personnel is being sadly neglected. As a result, Federal personnel is being developed to assume positions of responsibility and leadership. Most of the Federal training and development programs are available to state personnel but, for reasons known only to themselves, State Officials are not taking advantage of this opportunity. I believe the effects of this failure to upgrade state personnel will be evident when we wake up to the realization the Federal Government is furnishing the epidemiologists, diagnosticians and project leaders and the states are furnishing the peons. If the animal health experts in a state are all
Federal why, then, should the State Officials expect the livestock industry to look to them for leadership? Too often the Federal forces represent stability and know-how, and the state force the opposite. However, not all of this results from lack of training. Some results from the insatiable desires of politicians to play "politics" with state offices, no matter how remote the officials' obligations and responsibilities are from the ballot box.

Certainly none of us are beyond the need of continued education and training, and there is no question that we sorely need more trained State Regulatory personnel. For these reasons, we should be eager to cooperate with Federal officials in the development of training programs and to participate in them when offered the opportunity instead of complaining about "those damn Federally dominated work conferences and training courses." State officials can only blame themselves if they fail to develop their own personnel and the Federal agencies are forced to take over the State's obligations.

MODERNIZATION AND SIMPLIFICATION OF INTERSTATE MOVEMENTS OF LIVESTOCK

There is a great need to modernize our method of regulating, or perhaps a more appropriate expression "our method of restricting", the interstate movement of livestock. With the advent of preventive regulatory veterinary medicine, we should be able to discard the antiquated health certificate approach — with its 51 sets of regulations — for just one set of requirements and full utilization of the present miracles of communication. There is no valid reason why a rapid, accurate and practical program cannot be developed to record the free movement of livestock throughout the country. The challenge here is merely to put to use the tools already available.

NEED FOR IMPROVED COMMUNICATION AND PUBLIC RELATIONS

If our Animal Health Programs are to be effective we must have the understanding, respect and confidence of all facets of the livestock industry. This can be achieved only through improving communications between our organization and those representing industry.

Our failure to communicate will make future programs more difficult to implement. Every one should appreciate the urgent need for preventive animal health programs. Yet the greatest obstacle to implementing these programs will come from the livestock owner himself — the very person who has the most to gain if implemented and the most to lose if they are not. The livestock owner, as well as the organizations that represent him, are fearful of any program requiring the identity of their animals for traceback purposes and they will object to multi-screening tests being conducted on their animals at auction markets and slaughter houses. They distrust government, and especially animal health officials, so much they are blinded to the benefits of such procedures. Better public relations, communications and salesmanship, together with full utilization of common sense reasoning, will be necessary to overcome this major obstacle but it must be done soon as time is running out.
CONCLUSION

Since we’ve changed the name of our organization I have been trying to come up with some sort of simplified reference to it. I tried to use the first letter of each word but the letters didn’t flow as smoothly as our old reference to the USLSA. I then separated the letters phonetically into 3 syllables, U-SA-HA with the accent on the second syllable, pronounced U SA’ HA. That sounded pretty good – primitive — forceful, like it really meant something! I mentioned this problem to Dr. M. D. Mitchell from South Dakota, who in turn related my dilemma to one of his Sioux Indian friends who translated it for him. U SA’ HA in Sioux language means “one who leads.” Certainly no more appropriate word could be found to accurately express the United States Animal Health Association’s position in the past and in the role we hope it will play in the future. But unless we overcome our weakness and learn to work more closely and effectively with our allied organizations, our leadership as well as the organizations’ very life may come to an untimely end.

As stated in the beginning, the 1970’s will present the United States Animal Health Association its greatest challenge and its greatest opportunity. It will be a very crucial and trying decade for us. Our success or failure will depend on what we do or fail to do today.

I am confident the United States Animal Health Association, together with the allied organizations associated with us, will not only meet the challenge of the 70’s but will develop preventive animal disease programs and concepts that will protect the animal health of this nation for decades to come.

For some years our association has has a pleasant custom, originating I believe with our previous secretary-treasurer, Doctor Hendershott, of presenting its outgoing Presidents with a little token of the association’s esteem and appreciation. This token is in the form of a key bearing the association’s insignia, the recipient’s name, and the year of his presidency, and attached to a tie chain. It is my pleasure, as your president-elect, to present at this time to our incumbent President, Doctor O’Harra, this little token. So if you will come forward, Mr. President, it will be my honor to give you this expression of our gratitude.

For the last year or two, we have had some discussion about giving our outgoing president, in addition to this little piece of personal jewelry, a certificate attesting to his presidency and our appreciation of his efforts. We have had some difficulty in agreeing on the style and the format for this certificate, but we have finally had prepared what we feel is an attractive, and at the same time a dignified certificate. So, again, with our profound appreciation I present the first of these certificates to our outgoing President, Doctor O’Harra.

I must apologize for the fact that it is not framed. Our secretary tells me that he had every intention of bringing these to this meeting framed. He found, however, that the cost of lifting the signatures which we furnished him and having them transposed to the certificate by a mechanical process was very expensive and, therefore, he chose to have them signed here in person.

He has instructed me to inform you, Doctor, that he will be delighted to have the certificate framed as soon as he returns to his office, and will have it forwarded to you if you wish. On the other hand, if you prefer to take the certificate along as is and have it framed when you reach home, the secretary will be happy to have you send him the bill, which he will take care of promptly.
Now, if our immediate past president, Dr. John F. Quinn of Michigan, is in the audience, I would like for him to come forward. Doctor Quinn was presented with his key and tie chain at the last meeting in New Orleans, but the certificate was not then available. I have it here now, and it is my pleasure to present it to Doctor Quinn under the same conditions... the secretary will frame it, or if you prefer, Doctor, you can have it done when you reach home and have the bill sent to our secretary’s office. Doctor Quinn, if you please.
During the year, the Secretary and the office secretary, Mrs. Robert D. Blanton, attempted to provide the membership of the Association the services to which they are entitled and to bring an awareness of the activities of the Association closer to the membership.

Travel by the Secretary involved one trip to Las Vegas early in the year, at the request of the President, to attend the first meeting of the organizing committee of the College of Regulatory Veterinary Medicine, as set up in a resolution passed by the membership in New Orleans. The Secretary also attended a meeting of the State-Federal Relations Committee in Washington, as did the other members of the committee, and the office secretary. Mrs. Blanton has made two trips to Milwaukee to work out the details of the meeting with hotel personnel. I hope you will feel that her efforts were justified and this will be a most pleasant and successful meeting. We are also indebted to Dr. E. M. Ellis, who came to Milwaukee to assist in making the audio-visual arrangements, so that they would be both effective and satisfactory. Mr. Norman Powers also has made one trip to Milwaukee to work with Mrs. Blanton here in the hotel, so that we could have a smoothly functioning session here this week.

The Secretary, finding himself in Denver at the Interstate Milk Shippers Conference, took the opportunity to return via Oklahoma City to take a look at the hotel facilities there, accompanied by Dr. Brashear. Actually, this was the Secretary's second attempt, as he had made plans earlier in the year to return from the meeting of Livestock Conservation, Inc., at Sioux City by way of Oklahoma City to look at the facilities offered. On that first attempt, a blizzard intervened, so the trip had to be postponed until later during the year. The Secretary is happy to report that the Hotel Skirvin in Oklahoma City offers ample and excellent accommodations for our meeting, and we are scheduled there in October of 1971. Doctor Brashear also feels that the entertainment to be provided will be not only interesting but well worth the trip.

We still are somewhat hazy about our membership. In checking all of the plates that the Secretary received from the previous incumbent to the best of our ability, we have 863 paid members for 1969 and there are 283 to whom notices were sent that have not paid. This is comparable to our report of last year. So it would appear that we have somewhere in the neighborhood of between 860 and 875 active members. We should have twice that many, and the Association should devote itself and its energies to this end. There have been some complaints of members not receiving Proceedings. For this we extend our apologies, but we have done our best in this area. We have a considerable problem with returned Proceedings and dues notices because the addresses we have are currently incorrect. We must find a system that will encourage people who are our valued members to let us know
promptly when they move. Our mail is becoming voluminous and, if we are to continue to attempt to serve the membership as they appear to want to be served, it is only a question of time before additional personnel, either full-time or part-time, will be required, which suggests the need for additional income, and this means additional members. It also may mean a recommendation for another increase in our dues. The Secretary is not recommending an increase in dues for 1970, but you will all admit that in today's world with today's prices, ten dollars a year is extremely modest for active membership in this organization.

We are trying to speed up the date of distribution of our annual Proceedings. We have made some progress. This, too, can be improved.

Following considerable discussion by the Board of Directors during the year, the Secretary has attempted to change the format of our annual meeting. This is an experiment and subject to still further adjustment as the membership wishes. You will notice that our literary program starts Tuesday morning and continues each morning only for four sessions. We have set committee meetings during the afternoons, beginning with Monday and for four afternoons. We have tried to have as little conflict in committee meetings as is possible, but we could not design it so that there was none. We hope this meets with your approval; and if it does not, we hope you will suggest how it should be changed. This is your Association, it is your meeting, and it must be run the way you want it.

Insofar as our financial statement is concerned, our auditor has closed and balanced our books as of the thirtieth of September just passed, and we have improved our financial position in the sum of $2,775.40, which gives us a net worth of $37,911.04, as compared to our net worth of $35,135.64 as of September 30, 1968. A full and detailed financial statement will appear in the Proceedings at the end of this report. There are enough mimeographed copies of the statement here to furnish each member of the Executive Committee a copy. Any member who wishes a copy prior to the time it appears in the annual Proceedings of this meeting has only to write the Secretary's office, and a copy will be forwarded immediately.
W. L. BENDIX

UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR PERIOD OCTOBER 1, 1968 THROUGH SEPTEMBER 30, 1969

CASH BALANCE – OCTOBER 1, 1968:

Southern Bank and Trust Company
Richmond, Virginia (Savings) $5,266.98
Southern Bank and Trust Company
Richmond, Virginia (Checking) 776.21
Trevose Savings and Loan Association
Morrisville, Pennsylvania 1.00
Sandia Savings and Loan Association
Albuquerque, New Mexico 1.00

$ 6,045.19

INCREASED BY CASH RECEIPTS:

Individual Dues $8,639.00
Official Dues 5,000.00
Proceedings 4,295.10
Reprints 4,180.59
Registration Fees 6,825.00
Foreign Animal Handbooks 86.40
Interest Income 1,103.96
Local Arrangements 250.61
Federal, State, and F.I.C.A. Taxes deducted from Employees and have not been remitted 164.03
Check No. 108 not paid and re-deposited 21.25
Miscellaneous Revenue 371.66

$30,937.60

TOTAL BEGINNING BALANCE AND RECEIPTS $36,982.79
STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR PERIOD OCTOBER 1, 1968 THROUGH SEPTEMBER 30, 1969

DECREASED BY EXPENDITURES:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Meetings</td>
<td>$1,528.21</td>
</tr>
<tr>
<td>Printing</td>
<td>$11,363.36</td>
</tr>
<tr>
<td>Office Supplies</td>
<td>$427.36</td>
</tr>
<tr>
<td>Salary</td>
<td>$10,062.50</td>
</tr>
<tr>
<td>Social Security Tax</td>
<td>$378.43</td>
</tr>
<tr>
<td>Communications</td>
<td>$1,573.08</td>
</tr>
<tr>
<td>Travel:</td>
<td></td>
</tr>
<tr>
<td>Dr. John L. O’Harra, President</td>
<td>$1,020.06</td>
</tr>
<tr>
<td>Dr. Frank B. Wheeler, President-Elect</td>
<td>174.30</td>
</tr>
<tr>
<td>Dr. W. L. Bendix, Secretary-Treasurer</td>
<td>383.20</td>
</tr>
<tr>
<td>Ella R. Blanton, Office Secretary</td>
<td>600.39</td>
</tr>
<tr>
<td>Dr. E. M. Ellis, Local Arrangements</td>
<td>79.80</td>
</tr>
<tr>
<td>Miscellaneous Expense</td>
<td>$549.57</td>
</tr>
<tr>
<td>Office Equipment</td>
<td>$14.52</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>$7.42</td>
</tr>
</tbody>
</table>

Total Decreased by Expenditures: $28,162.20

CASH BALANCE – SEPTEMBER 30, 1969:

<table>
<thead>
<tr>
<th>Bank</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Bank and Trust Company, Richmond, Virginia, Checking Account</td>
<td>$198.90</td>
</tr>
<tr>
<td>Savings Account</td>
<td>8,397.03</td>
</tr>
<tr>
<td>Local Arrangements Account</td>
<td>222.66</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association, Morrisville, Pennsylvania,</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association, Albuquerque, New Mexico</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Total Cash Balance: $8,820.59
UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1968 THROUGH SEPTEMBER 30, 1969

REVENUE:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cash Receipts</td>
<td>$30,937.60</td>
</tr>
<tr>
<td>Less — Expenditures</td>
<td>28,162.20</td>
</tr>
<tr>
<td>Net Revenue over Expenditures</td>
<td>$ 2,775.40</td>
</tr>
</tbody>
</table>

NET WORTH — SEPTEMBER 30, 1969

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accounts Receivable</td>
<td>$ 5,225.56</td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Southern Bank and Trust Company, Richmond, Virginia</td>
<td>198.90</td>
</tr>
<tr>
<td>Checking Account</td>
<td></td>
</tr>
<tr>
<td>Savings Account</td>
<td>8,397.03</td>
</tr>
<tr>
<td>Local Arrangements</td>
<td>222.66</td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Trevose Savings and Loan Association, Morrisville, Pennsylvania</td>
<td>1.00</td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Sandia Savings and Loan Association, Albuquerque, New Mexico</td>
<td>1.00</td>
</tr>
<tr>
<td>Petty Cash Fund</td>
<td>25.00</td>
</tr>
<tr>
<td>Deposit — C&amp;P Telephone Company, Richmond, Virginia</td>
<td>100.00</td>
</tr>
<tr>
<td>Inventory — Supplies and Proceedings</td>
<td>2,483.37</td>
</tr>
<tr>
<td>U.S. Treasury Bonds</td>
<td>20,000.00</td>
</tr>
<tr>
<td>Furniture and Fixtures</td>
<td>896.52</td>
</tr>
</tbody>
</table>

Net Worth — September 30, 1969: $37,911.04

ANALYSIS OF CHANGE IN NET WORTH

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net Worth, September 30, 1968</td>
<td>$35,135.64</td>
</tr>
<tr>
<td>Increased by:</td>
<td></td>
</tr>
<tr>
<td>Net Revenue from Operations for fiscal year</td>
<td>2,775.40</td>
</tr>
<tr>
<td>ended September 30, 1969</td>
<td></td>
</tr>
</tbody>
</table>

Net Worth, September 30, 1969: $37,911.04

Henry H. Budd
Accountant
President O’Harra, convention delegates, ladies and gentlemen — with pleasure I welcome you and this outstanding organization to Wisconsin with its 8,700 lakes, its 20,000 miles of rivers and streams, its great lake seashores, its timbered highlands, kettle moraines, bristling cities and quaint little mining towns.

To this group, especially, I welcome you to our lush green farmlands where 2½ million dairy cattle reside, producing 16% or 18½ billion pounds of the nation’s milk supply annually.

From this southern gateway metropolis to the Indian Head Country of the North, the people of Wisconsin extend a warm welcome. We hope that many of you will linger with us to enjoy Wisconsin’s colorama, the blazing colors of our great oaks, maples, and many of the other varieties which contribute to the beauty of this state.

May I also extend greetings from our National Association of State Departments of Agriculture and express appreciation to Dr. O’Harra, Dr. Bendix and Dr. Quin for their contributions during our recent convention in Syracuse, New York.

Your organization and its contributions through the years have been extremely important to the Wisconsin Department of Agriculture and the agricultural interest we serve. While I may be new as the Secretary of our Department, I proudly indicate that I have served this agency since 1948 when I joined it as an Information Specialist. It is interesting to note that in that year 4 Wisconsin men were serving on USLSA committees.

It has also been interesting for me to note that during this past 21 years Wisconsin men have continued to be active on your committees and that the recommendations from these committees and in turn from your organization have often guided us as we have developed and strengthened our animal disease control and eradication efforts.

It was about that same period of time that my friend, Bill Knox, the distinguished editor of HOARD’S DAIRYMAN, as a member of your USLSA Brucellosis Committee said, “Brucellosis is without question the most important, most critical problem facing the dairy cattle industry today”. Twenty-one years later, the nation is on the threshold of completely eradicating this disease.

During this period of some two decades, it has been my privilege to observe your Association as an “action” organization, coming to grips with animal disease problems of tremendous proportions, coming forth with sound, valid, and practical recommendations. You are to truly be commended for this achievement.

Following the lead of your Association, Wisconsin, during the past 20 years, has been successful in its fight to eliminate Brucellosis, Scabies, Foot Rot, Scrapie,
Vesicular Exanthema, Hog Cholera and Pullorum from its livestock and poultry. Hopefully in the next 20 years, Tuberculosis, Rabies, Salmonellosis, Leptospirosis, Mastitis and other diseases of livestock will follow this path to extinction.

But this group, especially, recognized the continuing challenge of new diseases, new methods of control and eradication. Your Association, your recommendations, are as vital to us in state government today as they were back in the trying days of Tuberculosis and Brucellosis.

Because many of you are associated with state or federal government, may I remind you that here in Wisconsin we have the somewhat unique and extremely effective program of one State-Federal Animal Health Division under one Administrator, Dr. Arden Erdmann. The complete union of the state and federal resources has been responsible to a very large degree to these successes enjoyed in disease control and eradication.

We are very pleased to indicate that this program has also recently served as the pattern for our new State-Federal Meat Inspection efforts which you may have heard referred to in Washington or throughout the nation as "The Wisconsin Plan". Already we have completed one of its major phases as the Federal and State Meat Inspection offices, even out in our districts, have been combined. Additional phases will ultimately lead us to the same point as in our Animal Health program, namely one united State-Federal Meat Inspection staff.

While we have long believed that this is the most effective method of providing government service, we are especially pleased now to note the great emphasis which President Nixon and his administration is placing in decentralizing the federal government's role through new relationships which federal agencies are developing with their state counterpart. We sincerely hope that all of state government will accept this great challenge and opportunity.

May I invite all who may be interested in learning more about our State-Federal efforts in these two important areas to visit with Dr. Erdmann, Administrator of our Animal Health Division, or Dr. Ed Baker, Administrator of our Meat Inspection Division.

We in Wisconsin are both conscious and extremely proud of the contribution which Agriculture continues to make to the welfare of our state. We join with you in saluting the importance of Agriculture with its basic responsibility of food and fiber production to this nation and, in turn, to the world.

May the deliberations and achievements of your organization during this convention and throughout this coming year continue to provide the needed leadership which so many of us throughout this nation have come to appreciate.

Welcome to Wisconsin! May your convention truly be enjoyable and productive, and as you leave us may your hearts be filled with the joy and contentment of a job well done.
RESPONSE TO ADDRESS OF WELCOME

J. C. Shook, Harrisburg, Pa.

Mr. President, Secretary Wilkinson, members and guests of the U.S. Animal Health Association, it gives me a great deal of pleasure to respond to those warm words of welcome from Wisconsin. For some of us this is our first visit to your state and city. You can rest assured that we have heard of Wisconsin and of Milwaukee. Who of us do not recall the ads for a certain beverage that uses the slogan “The Beer That Made Milwaukee Famous?” Someone last night informed me that this was not right — that it was really Milwaukee that made beer famous. I presume that by the end of the week the membership of the U.S. Animal Health Association will be able to set the record straight on which came first. Those of us who follow football have certainly heard of your Wisconsin Badgers and everybody in the world has heard of the Green Bay Packers and their great football team. I know of no other football team in the world who can play flawlessly in -15° weather.

I am sure this group thinks of Wisconsin in the light of another one of its slogans — “America’s Dairyland.” Realizing that Wisconsin is an important industrial state we cannot overlook the fact that Wisconsin agriculture is a giant industry with an investment of nearly 7 billion dollars, an annual gross income of well over 1½ billion dollars. It is this industry that is so vital to the expanding population of the nation and the world. It is these people in agricultural production who are keeping up with the demand for food even though our population is increasing rapidly. These accomplishments were made possible through the progressive thinking of Wisconsin people in Agriculture. This achievement is even more striking when one realizes that there are fewer farmers and less land in farm production than there were 10 years ago. This fact is true, not only in Wisconsin but in many other agricultural states.

Wisconsin has been a long-time leader in the field of agricultural research. Many innovations in agricultural production and particularly in dairy production have originated in Wisconsin. Your state is still number one in the population of milk cows and in milk production, cheese production and the production of many milk products. Not only is your total production in dairy products and dairy cattle commendable, but the quality is also something that is not to be overlooked. Wisconsin dairy cattle, for example, have a nationwide and an international reputation. I can attest to this in Pennsylvania by the fact that 6,215 Wisconsin cattle were imported into Pennsylvania during the past year. Only Canada exceeded this number. In the field of animal health, Wisconsin has also been a long-time leader. They were the sixth major livestock and dairy state to be brucellosis free. They were one of the first states to be free of hog cholera. They can also be proud of the fact that when they gain these commendable goals they do not relinquish them and, as I mentioned previously, many of us look to Wisconsin and follow the plan of Wisconsin in up-dating and making our disease programs more effective. This is a testimony — not only to the progressive thinking of your regulatory officials but to the fact that your entire dairy and livestock industry have a sense of pride and a
determination to achieve their goals.

Wisconsin's leadership is also evident in the Federal-State cooperative meat inspection program. We are all finding that a great many adjustments are necessary to make these programs effective and efficient. Wisconsin has developed a plan for establishing closer cooperation between State and Federal officials and to eliminate duplication of effort.

It is apparent in the few examples which I have cited that you people in Wisconsin follow your State Motto — "Forward" very well, and that the song that all of us have learned to recognize over the years — "On Wisconsin" certainly fits the thinking of the people of your state. Time will not allow us to discuss in detail your richness in natural resources, in the field of education, manufacturing, and national leadership. We cannot put a dollar value on Wisconsin's total contribution to the entire United States and to our individual states during the nation's relatively short history. We can attest to the fact, however, that Wisconsin has been a leader — it has been a State which all of us have admired and respected. On behalf of the U.S. Animal Health Association, I want to again express our appreciation to you for your warm words of welcome and for your invitation to have our 1969 annual meeting in Milwaukee. Thank you.
PRESIDENT'S REMARKS

John L. O'Harra, D.V.M.

Members of the USAHA, distinguished guests, ladies and gentlemen — I have been privileged to serve as your President this past year — my term of office is now nearing a close. This has been a very rewarding experience, enhanced at times by a certain amount of frustration. In my remarks a year ago as President Elect, I outlined my thinking and I am pleased to report that the more essential parts of my program have been enacted, and all-in-all I feel that progress for the Association has been accomplished.

As a result of a lot of hard work and planning by the local arrangements committee and others, I am pleased to report that we have a substantial registration at this meeting. I believe one of the largest to date. Milwaukee has been a good host city and we have accomplished a great deal of work and at the same time been nicely entertained. We must continue to build our membership.

During this past year I have been privileged to represent the USAHA at the American National Cattlemen's Association meeting in Honolulu, the National Association of State Departments of Agriculture meeting in Syracuse, New York, numerous high level meetings in Washington, D.C. and in other areas. As a representative of this Association I have enjoyed the hospitality and respect of many groups.

I am pleased to report the American College of Regulatory Veterinary Medicine is in its final organizational stages. During the past year a great deal of work was accomplished by the Organizing Committee and I feel that this recognition of Regulatory Veterinary Medicine as a specialty is well under way. This Specialty Board will need the full support of the entire membership and others who are not members, but who will be strongly effected. The merits of board certification should establish Regulatory Veterinary Medicine as a definite specialty area and I am sure give it the recognition that it so well deserves. After all, a specialty group of Regulatory Veterinarians who were established by an Act of Congress many years ago are certainly past due for recognition.

The office of our Secretary has passed through the transition of exchange following twenty-five years and is now functioning on a high level. Dr. Bendix and Mrs. Blanton have done yeomens work in this area and the results of this meeting are only one example of the fruits of their labors. The Association is still facing the problem of a more permanent home for the Secretary's office with location, cost and many other factors to be of prime consideration. I personally thank Ella and Bill for making my term of President a much more happy and desirable period of time than it could have been otherwise. The Officers of the Association, the Committee Chairmen, all Committee Members and certain Members of the Association all deserve a high word of praise and a sincere thank you for their efforts expended and their contribution toward our welfare.

The American Association of Veterinary Laboratory Diagnosticians are now of age and during the past year have resolved some problems of administration and cooperation with the USAHA which I feel has been a worthwhile accomplishment.
The members of our society who take the responsibility for diagnosis of the diseases that we are responsible for, plus in many cases, the cooperation in the regulatory procedures, are our finest colleagues.

We have a balance of the meeting to complete, I would not feel it proper to take more of your time. I would like to repeat that it has been a most satisfying year serving as your President and I again wish to extend the heartiest thanks to all who have contributed to the Association during this past year. I am sure Dr. Wheeler, your President Elect, upon taking over the office of President, will experience much of the win some, lose some, contribute all that you can experiences for the welfare of the USAHA, that I have enjoyed during this past year. I thank you.
Resolution

WHEREAS, there has been a definite deemphasis placed on the teaching of food hygiene principles by some Veterinary Colleges in recent years, and
WHEREAS, there has been a definite increase in the need for veterinarians with this training, and
WHEREAS, the area of food hygiene should be considered as important an aspect of Veterinary practice as any other, and
WHEREAS, most livestock and poultry are used for human food.
THEREFORE BE IT RESOLVED that the United States Animal Health Association urge the Council on Education of the American Veterinary Medical Association to require the principles of food hygiene be emphasized to all students enrolled in the Veterinary Professional Course in the accredited Colleges of Veterinary Medicine.

Resolution

WHEREAS, trichinae in our pork supply constitutes a public health hazard and has resulted in discrimination against United States pork in foreign trade and has had an adverse effect on domestic pork consumption, and
WHEREAS, the present low level incidence of trichinae in United States swine makes its eradication practical and opportune, and
WHEREAS, an accurate, practical test has been developed with the cooperation and support of the swine industry, the packing industry, and the United States Department of Agriculture, and
WHEREAS, a plan for utilizing this test in a combined consumer protection and national eradication program has been prepared for presentation to the Secretary of Agriculture,
THEREFORE BE IT RESOLVED that the United States Animal Health Association support this national program for the eradication of trichinae.

Resolution

WHEREAS, the Wholesome Meat Act signed December 15, 1967, requires states to provide meat inspection programs for intrastate plants at least "equal to" federal inspection, and
WHEREAS, the Wholesome Meat Act requires an "equal to" status with federal plants, and does not provide for privileges granted federal plants, and
WHEREAS, Meat products are permitted entrance into the United States from foreign plants declared to be operating under conditions "equal to" federal inspection, and
WHEREAS, present laws and regulations prohibiting movement of state-inspection product from plants declared "equal to" constitutes a restraint of free trade, and

xxxix
WHEREAS, some states are approaching certification of an “equal to” status,
THEREFORE BE IT RESOLVED that the United States Animal Health
Association urge the Congress of the United States to amend the Wholesome Meat
Act to permit meat establishments under a state inspection program which has been
certified as “equal to” federal inspection, to ship meat and meat products in
interstate commerce.

Resolution

WHEREAS, it is the present policy of the United States Department of
Agriculture to reimburse states on a 50-50 basis for services performed at plants
operating pursuant to the Talmadge-Aiken Act, and
WHEREAS, inspection services performed by states in plants operating
pursuant to the Talmadge-Aiken Act are federal functions which should be carried
out by federal employees, and
WHEREAS, reimbursement to states by the United States Department of
Agriculture is inequitable and does not fully reimburse the state for the
performance of this federal function,
THEREFORE BE IT RESOLVED that the United States Animal Health
Association request the Secretary of Agriculture to change the schedule of
reimbursement to fully reflect the cost to the states for the services performed.

Resolution

WHEREAS, the enforcement of the Wholesome Meat Act and the Wholesome
Poultry Act will require a large number of trained personnel, and
WHEREAS, we must find ways of carrying out the provisions of these Acts
with the smallest number of personnel possible, and
WHEREAS, most states have passed laws placing the responsibility of the
enforcement of these laws under the Chief Livestock Health official, and
WHEREAS, the number of people needed at both the state and federal level
could be greatly reduced if the responsibility of the enforcement of the Acts were
done under a joint agreement between the Chief Livestock Official of each state
and the Veterinarian in Charge, Agricultural Research Service in the state.
THEREFORE BE IT RESOLVED by the United States Animal Health
Association: that the responsibility for the enforcement of the Wholesome Meat
Act and the Wholesome Poultry Act be removed from the Consumer and Marketing
Service and placed in the Agricultural Research Service and that the enforcement
of these Acts be accomplished under a joint memorandum of understanding between
the Veterinarian in Charge, Agricultural Research Service and the State Official in
Charge in each state. And be it further resolved: that a copy of this resolution be
sent the Secretary of Agriculture of the United States.

Resolution

WHEREAS, it has come to the attention of the Animal Welfare Committee
that legislation has been presented to Congress for the purpose of preventing the
WHEREAS, it is the purpose and intent of this organization to promote the health and general well being of all domestic animals in the United States;

THEREFORE BE IT RESOLVED that the United States Animal Health Association in annual session assembled on the 17th day of October, 1969, at Milwaukee, Wisconsin, do support the intent of this legislation as it pertains to the protection of all horses from such acts.

Resolution

WHEREAS, a high percentage of equidae in Puerto Rico and the U.S. Virgin Islands have reacted to the CF test for equine piroplasmosis (EP); and

WHEREAS, it has been proved by inoculation of blood from horses in both Puerto Rico and the U.S. Virgin Islands into recipient horses that both Babesia caballi and Babesia equi exist in both locations; and

WHEREAS, horses affected with EP and/or infested with Dermacentor nitens have been moved from Puerto Rico into several states;

THEREFORE BE IT RESOLVED that the Code of Federal Regulations, Title 9, be amended to require the following prior to equidae being moved to the continental United States:

1. All equidae be treated to cleanse them of ticks and upon arrival in continental United States be inspected, found free of ticks and receive a precautionary treatment with a permitted parasiticide.
2. Prior to moving from Puerto Rico or the U.S. Virgin Islands, all equidae be subject to CF tests for EP and handled as follows:
   a. Animals not positive to CF Test be allowed to move if otherwise eligible.
   b. Animals positive to CF Test with B. Caballi antigen be allowed to move interstate following approved chemotherapy.
   c. Animals positive to CF test with B. equi or both antigens be allowed to move interstate following recommended chemotherapy and further allowed by negative animal inoculation proving them to be free of EP.
3. All such treatments be at the owners expense, properly supervised, and certified by a veterinarian of the ANH Division, U.S. Department of Agriculture.

Resolution

WHEREAS, it is generally known that equine piroplasmosis (EP) exists in many countries of the world; and

WHEREAS, there is an extensive movement of horses from such areas into the United States; and

WHEREAS, there is evidence that horses imported from such areas have been found affected with the disease or infested with Dermacentor nitens ticks upon arrival in the United States;

THEREFORE BE IT RESOLVED that the Code of Federal Regulation, Title 9, be amended to require the following:

1. All equidae, except those born in Canada, including zebras, be freed of ticks
in the country of origin, be required to have a USDA permit prior to leaving the country of origin, and upon arrival in the United States be inspected, found free of ticks and receive a precautionary treatment with a permitted parasiticide.

2. Prior to being released for entry, such equidae be subjected to the CF test for EP and handled as follows:
   a. Animals not positive to CF test be allowed to enter if otherwise eligible.
   b. Animals positive to CF test with $B. caballi$ antigen be allowed to enter following recommended chemotherapy.
   c. Animals positive to CF test with $B. equi$ or both antigens be refused entry or be allowed to enter following approved chemotherapy and further followed by negative animal inoculation tests at owners expense and properly supervised and certified by a veterinarian of the ANH Division, U.S. Department of Agriculture.

Your Committee submits the following nominees for officers of the United States Animal Health Association:

For President: Dr. Frank B. Wheeler
For President-Elect: Dr. Jean V. Smith
For First Vice President: Dr. M. D. Mitchell
For Second Vice President: Dr. W. C. Tobin
For Treasurer: Dr. W. L. Bendix

Your Committee submits the following nominees for regional industry representatives: **Northeast:** Dr. E. S. Bryant of Maine, Dr. William Henning of Pennsylvania; **West:** O. Timm of California, Archie Wilson of Montana; **Central:** J.W. Bishop of Indiana, Ward Van Horn of South Dakota; **South:** Joe Finley of Texas, Jim Nance of Tennessee.
REPORT OF THE
VETERINARY BIOLOGICS COMMITTEE

Carl J. Norden, Jr., Chairman, Lincoln, Nebr.

Licensing Activities. During Fiscal Year 1969 (July 1, 1968, to June 30, 1969), the number of establishments licensed by the Veterinary Biologics Division dropped from 57 to 55. At the close, 1,174 product licenses and 9 import permits being added. Licensees presented applications for 35 new products to be evaluated.

Licenses now in effect cover 280 generic products, manufactured in a total of about 13,100 component serials. Licensed biologics prevent, detect, or treat 58 animal diseases.

Regulatory Operations. One hundred and forty-six inspections of licensed establishments were conducted. Product surveillance tests for purity and safety or for efficacy, or both, on about 61 percent of the serials produced under license were conducted. This resulted in rejection of about 137 million substandard doses.

In addition, approximately 5 million doses were rejected by producers by their own testing procedures. These figures spell out clearly the hazards associated with biological production. However, the overall compliance record is improving due to improved production procedures and strengthening of standards.

The causes for rejection on complete manufacturer tests are given in Chart I and check tests by VBD in Chart 2.

Concurrent Testing. Division check tests were successfully conducted on a concurrent basis for the first full fiscal year. Concurrent testing diverges from the previous practice of doing check tests any time after a product is manufactured - and therefore, frequently after it already is marketed.

Now, check tests are done at the same time as in-house quality-control tests done by the manufacturer, and as a result, licensees do not market any products until they get clearance from VBD. This change eliminates the confusion and incomplete returns inherent in actions recalling products from the market.

Improved Test Methods. During the fiscal year, six new Standard Requirements for testing biological products were issued. These included methods for testing the potency or efficacy of Rabies vaccine, killed virus, Rabies vaccine, modified live virus of tissue culture origin, and Clostridium novyi bacterin-toxoid; methods for detecting hemagglutinating viruses in live virus poultry vaccines; sterility procedures for diluent; and requirements for selecting primary tissue cultures for vaccine production cells to assure freedom from extraneous and adventitious agents. Also, 22 existing Standard Requirements were improved and revised. One new and one revised Supplemental Assay Method to help licensees carry out proper laboratory procedures were also issued.

Uniform Biologics Standards. Fifty four different types of biologics standards - references and laboratory reagents - to carry out more nearly uniform quality control operations are now being distributed by VBD. These included 24 new references and reagents developed during the fiscal year.

Our support certainly continues in this area. In fact, we also encourage more extensive testing of the standards and more attention given to maintaining their
reliability during a specified dating period.

**Improved Laboratory Procedures.** New laboratory tests for determining potency of biologics, including a special procedure for finding the potency of each of the components of mixed virus vaccines were developed. Safety tests were worked out for detecting microorganisms that may contaminate live virus vaccines or infect tissue culture cells used for vaccine production. Another new safety test provides means to determine if tissue cultures cause tumors. And a newly developed laboratory procedure aids identification of bacteria by gas chromatography.

**Stable Tissue Cultures.** Vaccine manufacturers using tissue cultures for growing viruses, frequently start new primary cell lines — tissue cultures taken directly from animals — for fear that extended propagation of the same culture would cause cells to be altered. Separate tests for extraneous viruses are required for each primary cell line, a real chore for the manufacturers' staff. This is a continuing program and much progress is being made.

VBD cytology unit is developing methods for using a single primary cell line for a long period of time. Instead of using succeeding generations of daughter cell lines to maintain the tissue culture, the original primary cell line is frozen in liquid nitrogen and small portions of the line are expanded when tissue cultures are needed for manufacturing.

VBD has stored an embryonic bovine kidney cell line since November 1967, and it still retains viability. VBD is now working on improved freezing methods.

**Seed Virus Concept for Potency Testing.** Meaningful potency tests for live virus vaccines ideally should be carried out in the host animal, that is, the same species for which the vaccine is intended. But doing potency tests in host animals for every vaccine serial is too expensive.

VBD is working with industry to develop potency tests for master seed stock to be done before master seed is processed into serials. Canine distemper and hepatitis vaccine potency tests in dogs using master seed stock are correlated with virus titrations of the seed, forming a basis for evaluating potency of resulting serials just by virus titration in the laboratory. This concept is now being adapted to other live virus vaccines.

**Statistically Valid Sampling.** VBD has done preliminary studies with Biometrical Services of ARS on blackleg disease of cattle to determine the validity of existing potency test. The results provided the basis for doing a statistically meaningful test by means of a sliding scale that allows the highest quality products to be evaluated with the lowest number of test animals.

VBD is now categorizing other inactivated biologics to determine the proper number of test animals needed for statistically valid potency tests.

**Level of Antibiotics.** The permitted levels of antibiotics in live virus vaccines have been established during the year. To date, they appear workable. However, at the present low levels, continued experience is needed to determine the effect.

**Sterile Live Virus Vaccines.** Live virus vaccines which are subject to many manipulations in preparation and use of living tissue in their cultivation are quite
easily subject to minor contamination by environmental bacteria or fungi. Thus very low levels of bacterial contaminants have been permitted in the final product. Now, there is interest expressed in having the VBD require all such products to be sterile. Producers of these products agree that this is not impossible but is very impractical (or unnecessarily costly) due to an unwarranted waste of safe and effective vaccines. The unwarranted increase in cost would have to be borne by the uniformed user of such products. The VBD is contemplating issuance of proposed requirements for sterility of live virus vaccine, first for those which are injected parenterally.
TABLE I

QUALITY CONTROL ACTIONS FOR VETERINARY BIOLOGICS
(July 1, 1968 to June 30, 1969)

<table>
<thead>
<tr>
<th>Products by Animals for Which Intended</th>
<th>Amount Manufactured</th>
<th>Amount Marketed</th>
<th>Amount Rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serials</td>
<td>Doses (Millions)</td>
<td>Serials</td>
</tr>
<tr>
<td>Poultry</td>
<td>1,420</td>
<td>9,146.0</td>
<td>1,321</td>
</tr>
<tr>
<td>Swine</td>
<td>1,049</td>
<td>77.6</td>
<td>997</td>
</tr>
<tr>
<td>Cattle</td>
<td>6,619</td>
<td>347.0</td>
<td>3,412</td>
</tr>
<tr>
<td>Sheep</td>
<td>140</td>
<td>11.0</td>
<td>116</td>
</tr>
<tr>
<td>Horses</td>
<td>390</td>
<td>6.3</td>
<td>317</td>
</tr>
<tr>
<td>Dogs</td>
<td>2,994</td>
<td>55.1</td>
<td>2,783</td>
</tr>
<tr>
<td>Cats</td>
<td>283</td>
<td>4.1</td>
<td>265</td>
</tr>
<tr>
<td>Other*</td>
<td>205</td>
<td>22.3</td>
<td>196</td>
</tr>
<tr>
<td>TOTAL</td>
<td>13,100</td>
<td>9,669.4</td>
<td>12,407</td>
</tr>
</tbody>
</table>

*Other includes Fox, Mink, Rabbits, Chinchilla.
CHART 1

CAUSES FOR REJECTING VETERINARY BIOLOGICS
(July 1, 1968 to June 30, 1969)

Lack of Sterility and Potency (2%)
Lack of Sterility (35%)
Lack of Potency (48%)
Other Deficiencies (15%)
Licensees' Quality Control (Unsatisfactory Serials Only)

CHART 2

Lack of Sterility (47%)
Lack of Potency (52%)
Lack of both Sterility and Potency (1%)
Veterinary Biologics Division Check Tests (Unsatisfactory Serials Only)
REPORT OF COMMITTEE ON INFECTIOUS DISEASES OF SHEEP AND GOATS

M. D. Mitchell, Pierre, South Dakota, Chair; J. L. Hourrigan, Hyattsville, Maryland, Co-Chairman; C. C. Beck, East Lansing, Michigan; G. L. Crenshaw, Davis, California; W. J. Hadlow, Hamilton, Montana; W. A. Hickman, Pierre, South Dakota; Blaine McGowan, Davis, California; R. E. Simmons, Boise, Idaho; T. B. Snodgrass, Dallas, Texas; O. H. Timm, Dixon, California; Ward Van Horn, Buffalo, South Dakota; J. B. Henderson, Austin, Texas.

During the past year your Committee has considered several health problems which cost the sheep industry heavy financial losses. After much deliberation, your Committee has come up with the following recommendations:

BLUETONGUE

Blue tongue is a costly disease of the sheep industry of the United States and due to its multiple strains and variable viremia in affected animals continues to create problems in diagnosis. The disease is also being recognized clinically with increasing frequency in cattle and is apparently developing strains that are more virulent in this species.

During calendar year 1968, 10 outbreaks of bluetongue in sheep were diagnosed clinically in the states of Arizona, Colorado, Indiana, and Oregon. Yet in only 1 flock in Oregon was the diagnosis confirmed by virus isolation from 8 attempts in Colorado, Indiana, and Oregon. During the same period, 25 outbreaks of bluetongue in cattle were diagnosed clinically in the states of California, Florida, New Mexico, Oregon, and Texas. Here again only 9 virus isolations were obtained from 23 attempts in California, Florida, New Mexico, and Oregon.

These data confirm the need for continued study into all facets of bluetongue in cattle, sheep, and other animal populations of the United States. In recognition of the problems involved the Animal Disease and Parasite Research Division's Animal Disease Research Laboratory (ADRL), Denver, Colorado, in cooperation with the Animal Health Division, are presently conducting a cooperative study to determine the reservoirs of the bluetongue virus, strains of the virus present in the United States, Modes of transmission of the disease, clinical syndromes in cattle and sheep, efficacy of available vaccines, carrier state of affected cattle, sheep, and/or other animals, and the development of practical diagnostic test(s) for the disease. This study would be greatly enhanced by early requests for the services of the ADPR Laboratory and ANH Bluetongue Epidemiologist, Denver, Colorado, in cases of suspected bluetongue in cattle and sheep.

Our Committee supports the resolution of the Committee on Infectious Diseases of Cattle that increased funds be made available for accelerating bluetongue research at the ADPR Laboratory in Denver with particular emphasis being given to cattle.

SHEEP FOOT ROT

The Committee discussed sheep foot rot at length and recognized that in large areas of the country, the disease is of major economic importance.
The subcommittee on incidence and evaluation of losses from foot rot had completed one survey and hope to develop more meaningful data by the middle of 1970. We urge continued research to develop more practicable eradication procedures.

The Committee agreed to amending 9 CFR, Part 713, to delete the words, "While in transit or upon arrival at a feed lot, stockyard, or marketing center" thus correcting an inequity and also providing for movement of animals with certain diseases as specified in Part 713, if found affected other than while in transit or at concentration points.

NEONATAL LAMB LOSSES

In consideration of losses of 15-20% of the lambs in certain areas of the United States, the Committee voted that research on neonatal mortality be encouraged and supported.

OVINE VIRAL ABORTION

The Committee continued its recommendation of last year which was that State and Federal regulatory bodies support the testing and production in the United States of an effective vaccine.

RAM EPIDIDYMİTIS

The Committee recommends that State and Federal regulatory bodies give continued support to the work being conducted in California and Idaho and at the laboratories at NADL and Mission, Texas, and that more work be done to identifying the specific causes of the disease.

SCRAPIE

Scrapie was reported in 8 flocks in 4 states during Fiscal Year 1969, 2 less than the 10 outbreaks that occurred during the previous year. The 8 outbreaks involved 7 Suffolk flocks and 1 outbreak in an Cheviot flock. The number of flocks under surveillance has now risen to 296, up 26 flocks from last year. The increase in flocks under surveillance are the result of tracing of sales from 2 large Suffolk source flocks, 1 each in California and Pennsylvania.

The 8 outbreaks occurred, 1 each, in Sacramento County, California; Macon County, Illinois; Tioga and York Counties, Pennsylvania; and, 2 each, in Vanderburgh County, Indiana; and Lancaster County, Pennsylvania.

Three of the outbreaks were reported by veterinary practitioners; 3 were reported by the flock owners; and 2 were found when tracing bloodline and/or exposed sheep.

All bloodline and nonbloodline exposed sheep in the 8 infected flocks and their source flocks were slaughtered with the exception of 28 bloodline sheep from the California infected flock and its source flock which were taken to Mission, Texas, for use in the Scrapie Field Trial. All other bloodline sheep sold from infected and source flocks were slaughtered and in addition the immediate progeny
of bloodline sheep sold from a Pennsylvania source flock were also slaughtered. This Pennsylvania flock was the source of 3 Pennsylvania and 1 Maryland outbreak. Nonbloodline exposed sheep sold from infected and source flocks in California, Illinois, and Pennsylvania are being traced and placed under 42 months surveillance. Indiana was unable to identify bloodlines, therefore, all exposed sheep sold from the infected and source flocks were slaughtered.

**SCRAPIE FIELD TRIAL — MISSION, TEXAS**

The Scrapie Field Trial has been underway since November 1964 (58 months); however, most of the animals taken to Mission for this study have been obtained since March 1965.

On infected premises No. 3 there have been some 1,797 animals either born there or purchased from 10 states and New Zealand. The bloodlines sheep brought into this flock are of the Cheviot, Hampshire, Montadale, and Suffolk breeds and were purchased from flocks involved in 27 outbreaks in California, Indiana, Illinois, Maryland, Missouri, New York, Texas, and Virginia. These sheep represent 21 bloodlines in which scrapie has been found in the United States. The nonbloodline animals in the flock are Hampshire, Rambonillet, Suffolk, and Targhee sheep breeds; and Angora, Nubian, and Toggenburg goat breeds. These animals were purchased from Oklahoma, Montana, Texas, Wyoming flocks and from New Zealand and represent 20 individual bloodlines. To avoid criticism of the results should any of the United States nonbloodline, previously nonexposed Suffolk sheep used in the trial develop scrapie, 24 purebred Suffolk (4 rams and 20 ewes) were imported from New Zealand on May 19, 1969, for use in the field trial contact studies. None of these 24 imported sheep or their descendants will be sold. They as well as all other animals used in the study will be destroyed during the course of the study or at the completion of the study.

None of the nonbloodline, previously nonexposed, sheep or goats have been confirmed scrapie. However, 4 Hampshires, 2 rams and 2 ewes, 1 ram and one ewe purchased, and 1 ram and 1 ewe born at Mission, have been observed to show early signs indicative of scrapie. The 2 purchased Hampshires are 55 months of age, and the 2 born at Mission, 31 months of age.

Sixty-five cases of scrapie have been diagnosed on infected premises No. 3 from December 1964 through July 1969. These cases have occurred in the following categories:

<table>
<thead>
<tr>
<th>CATEGORIES</th>
<th>NUMBER OF SCRAPIE-AFFECTED SHEEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Dams of affected sheep</td>
<td>2</td>
</tr>
<tr>
<td>B. Sires of affected sheep</td>
<td>2</td>
</tr>
<tr>
<td>C. Progeny of affected rams and ewes</td>
<td>2</td>
</tr>
<tr>
<td>D. Progeny of affected ewes</td>
<td>13</td>
</tr>
<tr>
<td>E. Progeny of affected rams</td>
<td>3</td>
</tr>
<tr>
<td>F. Full-siblings to affected sheep</td>
<td>5</td>
</tr>
<tr>
<td>G. Half-siblings to affected sheep via the sire</td>
<td>20</td>
</tr>
<tr>
<td>H. Grandprogeny of sires of affected sheep</td>
<td>6</td>
</tr>
<tr>
<td>I. Exposed mixed breed dairy goat</td>
<td>1</td>
</tr>
<tr>
<td>J. Field suspects held for observation</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>
The Scrapie Field Trial has demonstrated that when bloodline exposed sheep are held under observation many of them will develop scrapie. Scrapie losses in these bloodlines are significant and can run as high as 27% per year and can affect as many as 40 to over 60% in certain bloodlines. Sex does not appear to play a part in the incidence of scrapie, as a wether, a hermaphrodite, and proportionally equal number of rams and ewes have been affected. The study has also shown that when animals from certain infected or source flocks are held under observation, scrapie may continue to occur in animals from these flocks for one or two years and then decline rather rapidly or cease. However, in one bloodline where this has occurred, second generation progeny is now beginning to develop scrapie.

A rather severe outbreak of bluetongue has occurred in the Mission flock. The disease started rather slowly in the 1969 lamb crop in late June and gradually worked into the older rams and ewes. The disease has been complicated by extremely hot weather and a secondary pneumonia and some 74 animals have died. Treatment has consisted of combiotic for individually affected animals, aueromycin in the feed and vaccination of nonaffected animals with a modified live-virus vaccine of chicken-embryo origin. This has stopped the spread of the disease, but some deaths are still occurring in convalescent cases. The strain of virus has not yet been identified by the Denver, ADPR, Laboratory. The source of the outbreak has not been determined, however, it is suspected it was imported with a group of bloodline western sheep purchased in February 1969.

The Committee voted to support the continuation of the Scrapie Eradication Program and to urge continuation of the Mission Field trial and cooperation with active scrapie research workers.
Health certificates for animals are designed to serve two purposes: (1) to expedite movements of the animals from place to place and (2) to protect against the introduction or spread of communicable animal disease. The form and substance of the certificate changes from time to time as changes occur in the incidence of disease and in general programs of prevention, control, and eradication.

The members of this Association have given attention to the wording and the procedures for handling health certificates for many years. Many beneficial actions have been taken to achieve uniformity. For example, quite a number of the states have adopted the recommendations of the Committee on Laws and Regulations which appeared in the Proceedings of the 68th Annual Meeting of the Association.

The certificate usually calls for an inspection by a veterinarian and a verification of certain specified tests and vaccinations. Signature of an accredited veterinarian, with verification of his status by a State or Federal official, is usually necessary.

There are a number of problems that face accredited veterinarians in handling these certificates. Among the most important are:

1. Some certificates contain an unqualified statement that the animals are free of communicable disease. This calls for the veterinarian to make a statement which he probably cannot verify. Most forms avoid this by including qualifying language, such as “free from evidence of communicable disease.”

2. Many certificates contain a statement that the animals have not been exposed to communicable disease, but there is no qualifying wording such as “evidence of” nor is there any limitation as to time, such as within 60 days next preceding the date of inspection. Perhaps more importantly, there is no commonly accepted criteria for determining “exposure.” For example, does passage through a general stockyard constitute such exposure? Does contact with known reactors to brucellosis or tuberculosis constitute exposure? In either case, if it is assumed that there has been exposure, how much time must elapse before the animals may be considered free of exposure for purposes of certification?

3. Many certificates require that the accredited veterinarian state that he is familiar with the requirements of the place of destination. This seems to call for a breadth of knowledge far beyond the specifics of the document and, as a practical matter, knowledge that is not available in detail to the accredited veterinarian. As most of you will agree, it is extremely difficult for State and Federal veterinarians who are working with these subjects every day to keep abreast of all the requirements of the various jurisdictions within this country. When one adds to this the requirements of a multitude of foreign countries, the task of acquiring the knowledge required to sign this statement on the certificate really assumes gigantic proportions.

4. Some certificates use the words “personally inspected,” some use the word
"inspected," and some say "examined." Apparently each of these terms is intended to convey the same meaning. However, there has never been any uniformity of thought in the interpretation of the terms and, once again, the accredited veterinarian is without guidelines.

The AVMA has become concerned with this problem through the operations of the AVMA Professional Liability Insurance Trust. In the review of claims made against veterinarians for alleged malpractice, we find instances in which the certificate signed by the veterinarian for the movement of animals has been the key document to support the allegations of the claimant. Under such circumstances it is not always persuasive to assert that the use of health certificates is not intended to be a guarantee against all manifestations of disease among the animals involved. The attorneys in the case and the judge and jury are concerned with the wording of the certificate. The fact that thousands of other certificates of similar wording are executed every year does not help very much in the defense of the veterinarian if the statements on the certificate are found to have been inconsistent with the health status of the animals, even though the particular disease situation was discovered much later and could not have been ascertained at the time through ordinary inspection procedures.

The AVMA Council on Public Health and Regulatory Veterinary Medicine has given attention to this matter. We are proposing that this association, perhaps through its Committee on Laws and Regulations, join with the AVMA Council on Public Health and Regulatory Veterinary Medicine and the AVMA Professional Liability Insurance Trust in making a thorough review of the form and requirements for the use of health certificates and to make recommendations for improvement. If the association looks favorably upon this suggestion, we will be glad to participate in arranging for an early meeting of all those concerned.

Thank you, Mr. President, for this opportunity to meet with the members of this Association and to discuss an important problem with you.
REPORT OF
THE COMMITTEE ON LAWS AND REGULATIONS

Sheraton-Schroeder Hotel
Milwaukee, Wisconsin
Oct. 12-17, 1969

Dr. G. B. Rea, Salem, Oregon, Chairman; Dr. L. N. Butler, Phoenix, Arizona; Dr. Dean E. Flagg, Bismark, North Dakota; Dr. J. F. Huddelson, Topeka, Kansas; Dr. T. A. Ladson, College Park, Maryland; Dr. E. R. Mackery, Hyattsville, Maryland; Dr. G. A. Martin, Washington, D.C.; Dr. D. L. Smith, Indianapolis, Indiana.

Subject material for the Committee on Laws and Regulations this past year consisted of one new problem and a carryover from last year of three subjects which were referred to the Animal Health Division (ANH) and Meat Inspection Division of Consumer & Marketing Service (C&MS) for consideration and report.

One of the subjects to be reported on after review by the ANH was that of establishing quarantine facilities on some "Off Shore" island, for which much need has been shown, and which was supported by the USAHA last year. Progress on the project has proceeded to the extent that 4 identical bills to provide for these facilities have been presented to Congress. They have been referred to the respective Agriculture Committees of both houses which have in turn requested the U. S. Department of Agriculture for recommendations concerning enactment of this legislation. Our Committee urges strenuous support for this legislation and for the appropriation of funds to implement it.

The second subject referred to the ANH by last year's Committee had to do with the indemnification of animals slaughtered due to the outbreak of exotic or foreign diseases when in interstate channels of trade. It was felt by your Committee that the financial burden thus incurred would not only be greater than most individual states could afford, but, because of the potential variation in origin of these animals, it would be grossly unfair to any single state, which by accident of location might be the focal point of such an outbreak. An illustration of such areas would be a major livestock market, feedlot or collection point. At that time — October 1968 — The Emergency Disease Staff of the ANH had recommended that the present regulations concerning indemnity payments be changed so as to allow 100% federal reimbursement under certain specified circumstances. We have just these past few days been informed that these changes have not yet been adopted. We view this situation with alarm and urge the Department of Agriculture to make hast in revising the regulations pertaining to indemnification of animals under these circumstances.

The question referred to the C&MS with regard to the identification of carcasses during the slaughter process has been answered and implemented.

As a result of previous discussion concerning the need for regulation of interstate movement of zoo type animals, the Committee now recommends that the ANH and individual members of this association (USAHA) pursue the subject with both Public Health and Fish and Game officials at both the state and federal level with the thought in mind of determining the need for disease control measures.
prior to interstate movement of this type of animal. Further, the Committee recommends that representatives of the U. S. Fish and Wildlife Department and the U. S. Public Health Department be invited to participate in this discussion at the next annual meeting of the USAHA. The Committee members have asked their chairman to make a survey of the states to determine where the authority exists for the control of diseases in zoo type animals.

A new subject which concerns the liability of the accredited veterinarian in preparing and signing health certificates for interstate movement of livestock was considered. Consultation with a representative of the AVMA indicated serious hazards as pertain to the presently used statements of certification. Since the Committee had not had adequate opportunity to study and discuss the intricacies of the problem sufficiently, it was decided that a meeting with representatives of the AVMA Insurance Trust Officers and the Council on Public Health and Regulatory Medicine of the AVMA would be held in the near future and recommendations developed for consideration at the next annual meeting of the USAHA.

Mr. Chairman, this constitutes the report of the Committee on Laws and Regulations. I recommend its adoption.
REPORT OF THE COMMITTEE ON
REGULATORY VETERINARY MEDICAL RESOURCES


The Regulatory Veterinary Medical Resources Committee met with the State-Federal Relations Committee and Federal officials in Washington, D. C. on February 10, 1969, at the request of Dr. E. E. Saulmon.


Dr. E. E. Saulmon explained to the Committee that an indepth study had been made of the organizational setup of the field programs and that considerable thought had been given to how this organizational setup could be improved upon. He felt that this study was necessary in that the down-the-road testing of cattle for brucellosis had been largely replaced by screening processes and epidemiological studies. He stated that the programs now require a more highly trained professional person and that this should result in an upgrading.

It was his suggestion that the separate positions of District and Area Veterinarian as well as the GS-12 Epidemiologist, be abolished and that a combination of several positions be established. In line with the new titles recently established by the U. S. Civil Service Commission the title would be Veterinary Medical Officer. This position would carry a GS-12 rating. This Veterinary Medical Officer would be a person who had received special training in at least one field of diagnostic work such as hog cholera, vesicular diseases, etc., in addition to the required basic orientation in epidemiology. In some cases, he may be trained in more than one of these specialty fields. The requirement for supervising at least six employees in order to be a GS-12 has been replaced by the requirement for more specialized knowledges and skills. He would be in charge of all regular Veterinary Medical activities within his assigned area and would be directly responsible to the State and Federal offices. This organizational setup would furnish a more highly trained person who was in direct contact at the livestock owner level. The Veterinary Medical Officer could supervise all disease control activities, all of the inspection activities, all of the port activities and all other livestock disease control functions within the area and the determination of the actual assignment would be on the basis of workload rather than the functional separation. The rating of GS-12 would give him a higher rating than the District Veterinarian now has. From time to time, field personnel would be selected for V.A.D.P. training and those selected would, after completing this training, be reassigned to higher level positions.

It was Dr. Saulmon's hope that the organizational problems could be worked out on a State-by-state basis and that State personnel could be upgraded in the same manner. Training provided for Federal personnel would be made available to State employees.

The Committee has stated in previous reports that the best working
relationship between the State and Federal authorities has been accomplished when states were in position to furnish at least 50% of the cost of the programs and when the state was in position to furnish its equal share of personnel to accomplish the job at hand. Again the Committee would like to request that all state officials give careful consideration to this fact and that sufficient state personnel be employed and that full advantage be taken of the various training programs so that these state employees will be as fully capable of taking over their responsibility as are the federal employees.

New problems will be created in the future in obtaining sufficient funds to carry out our various state and federal programs. These problems are multiplied by the fact that an ever decreasing number of our population is engaged in the production of livestock and poultry. For that reason, we would like to call the attention of all state and federal officials to take a closer look at what might be our role in the control of animal and poultry diseases within the next five to ten years. This Committee plans to make a thorough study of what might lay ahead and will attempt to lend its efforts toward what might be some solution before these problems become too much of a reality. The Committee should concern itself with what kind of organization might be needed and how this organization might be established in order that we might be better equipped to meet the changes as they occur.

The passage of the Wholesome Meat Act and the Wholesome Poultry Act has placed further stress on our Veterinary medical resources. As more and more states become involved in the enforcement of these two acts, we begin to more fully realize that there is not available enough qualified and trained personnel to assume the responsibilities of carrying out the purposes of these two acts if we continue to try to accomplish this end with the present organizational structure under which these two acts are being administered. It is the feeling of the Committee that it would be far more economically feasible to carry out the intent of these two laws if the responsibility for carrying out these laws were placed under the same agency that are presently involved in carrying out all of the animal disease eradication and control programs. In other words, it is felt by the Committee that state laws should be so written that the responsibility of the inspection of meat and poultry be placed under the Chief Livestock Sanitary Official of the State and that the enforcement of the Wholesome Meat Act and the Wholesome Poultry Act be accomplished under a joint agreement with the Veterinarian in Charge, Agricultural Research Service, and the Chief Livestock Sanitary Official as are all the animal disease eradication and control programs at the present time. This would mean that the enforcement of the Wholesome Meat Act and the Wholesome Poultry Act be removed from the Consumer and Marketing Service and placed in the Animal Research Service. The Committee wishes to present a resolution on this subject to this organization for its consideration.
The Import-Export Committee met at 1:30 P.M. Tuesday afternoon. There is attached a list of those attendance. Chairman Quinn advised the group that this was a new designated Committee of the USAHA and it would be permanent in nature for such period as deemed advisable and necessary. Dr. Quinn then read the contemplated functions and responsibilities:

1. To find the best way to protect the United States from dangerous animals and animal products.

2. To recommend a system to provide maximum protection and how to finance it.

Dr. Quinn also explained the nature and reasons for sub-dividing the Committee into the following designated Sub-Committees: Sub-Committee on Domestic Animals & Birds; Sub-Committee on Animal Products and Byproducts; Sub-Committee on Wild and Endangered Species of Animals & Birds.

There was furnished to each member the 1961 Task Force report. The recommendations of this report were reviewed and a question asked, “How many of them had been brought into being?” In explanation, it was indicated that some of the items and situations as outlined in the report had been changed due to lapse of time.

Following this, the meeting was then opened for general discussion, with the following responses:

Regarding the importation of exotic animals, birds, etc., three agencies are involved, USDA, Public Health and Interior. The total volume at the present time is very sizeable and is increasing.

Questions were asked relative to prohibitory actions that can be taken to safeguard this country against foreign animal diseases. It was explained that there are two routes, Secretarial authority and Animal Quarantine authority. These particular authorities are for the most part related to F&M, Rinderpest, etc., and are related to those diseases which may be dangerous to domestic animals of this country. An example; of an area not covered, in that there are no requirements in regard to rhinocerous, since it has not been established that they carry a disease dangerous to domestic animals.

Regarding financing present activities the subject of landing fees was discussed and this is the only country not utilizing this system. Such establishment would no doubt create some degree of opposition from the transportation companies.

A question was asked regarding utilization of Canadian facilities and the present procedures were explained.

Dangers associated with the increase of foreign visitors to this country was discussed, and the following illustration was used, i.e., 202 million visitors as related
to 200 million population. The volume at some ports is increasing to the point where there is no sufficient space for initial inspection, so off-port inspection sites are being authorized by Customs.

The one-stop system at airports was explained in the context of the volume of people and desirability of moving things and people faster; another point is the increased use by the airlines of containerization in cargo shipments.

Questions were asked relative to the present status regarding the possibility of an off-shore quarantine, station; there is no specific action as to the date; however, it is still in the discussion process. It was suggested that perhaps consideration should be given to another survey trip due to the span of time from the 1961 report. Consensus of the group—not needed at this time.

Summary

1. The Committee was advised of the contemplated functions and responsibilities.
2. The overall Committee was broken down into three specific Sub-Committees.
3. The general opinion that these Sub-Committees should meet in the interim period between the annual USAHA meetings and each Committee would meet if possible in connection with their own group needs, with the Sub-Committee Chairman to determine need, time and locations.

Sub-Committee on Domestic Animals & Birds
- H. G. Wixom, Chairman
- C. L. Campbell
- Paul C. DeLay
- Frank Harding
- James R. Hay
- James B. Henderson
- John R. Landridge
- Robert Rumler

Sub-Committee on Animal Products & Byproducts
- J. C. Shook, Chairman
- E. L. Brower
- J. J. Callis
- Grant S. Kaley
- W. W. McMichael
- A. R. Miller
- W. L. Sulzbacher

Sub-Committee on Wild & Endangered Species of Animals & Birds
- T. H. Reed, Chairman
- R. A. Bankowski
- George E. Cottral
- L. J. Goss
- Gus Griswald
- R. E. Omohundro
- John Richardson
- Charles C. Schroeder
- C. L. Smith
- Robert Willson

The following non-Committee members were in attendance:
- Olin Henry Timm
- E. W. Christeck
- J. L. Wilbur
- J. L. Hourrigan
- G. W. Spangler
- J. K. Atwell
- W. B. Henschele
- R. L. Knudson
GRADUATE STUDY FOR REGULATORY VETERINARIANS

Calvin W. Schwabe, D.V.M., M.P.H., Sc.D.

In the long run the utility of any profession is probably determined solely in terms of the lasting impact which its activities have upon society in the promotion of man's wellbeing. By this criterion veterinary medicine obviously has been, and still is, a profession of the greatest social utility. An objective appraisal of veterinary medicine's social significance would almost certainly reveal that our professions' truly lasting benefits — and those for which we can be most justifiably proud — have been realized through remarkably successful efforts to prevent and control, and sometimes even to eliminate permanently from entire countries, important diseases of domestic animals. Many of these diseases threaten directly the health of man and all of them markedly affect man's wellbeing by contributing substantially to deficits in protein foods of the highest nutritional quality, as well as to locally important losses of draft animals and valuable animal products.

It goes almost without saying, therefore, that the activities of those veterinarians who are most closely and directly associated with these programs of livestock disease prevention, control and eradication have had — and now have — an unusually high social value. The question I shall explore with you this morning is, consequently, a very important one not only for our profession but also for society at large. It concerns some of the desirable qualifications which veterinarians should possess for the continued performance of such important and highly specialized veterinary activities as those we have just mentioned.

Before we move on to this question, we might first just note in passing that virtually all new activities by any profession are at first undertaken by individuals who are not specifically trained to do so. Gradually, however, as a distinctive discipline and tools evolve so do recognized programs of training which cater specifically to these needs. Thus, in practically all laboratory-based veterinary research activities and in many areas of veterinary academic and clinical activity it is now universally appreciated that training to the level of the professional DVM degree is not in itself sufficient for the responsible performance of these highly specialized functions.

What then is the present situation with respect to our own field of livestock disease control? This field is certainly not one which can any longer be considered "new". Rather it is one in which a great deal of experience has been gained in tasks successfully accomplished. In addition we have made some mistakes in the past which, if we do not attempt to hide or bury them, we can learn a great deal from and we can profit from in the future. It behooves us to be fully and critically aware of these past efforts — for veterinarians have truly pioneered in population medicine in the development of many completely original approaches to disease — approaches such as the mass testing or screening of populations, vector control, the

Department of Epidemiology and Preventive Medicine, University of California, Davis 95616. Presented at the 97th Annual Meeting of the U.S. Animal Health Association, Milwaukee, Wisconsin, October 16, 1969.

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mass immunization and mass treatment of populations and most exciting of all, the revolutionary concept of disease eradication. Informed public health officials readily acknowledge their debt to our profession for these innovations and others which we in veterinary medicine have developed and which they now also employ.

I wonder, however, if we veterinarians are still really pioneering in population medicine at the rate we once were. If not, a question we must in all honesty ask ourselves is how well or how poorly at the present time does the DVM degree — even supplemented as it sometimes is by the more usual types of private practice experience — fully qualify a veterinarian to enter different types of work in our field of preventive veterinary medicine. For example, is the average young veterinarian able to investigate scientifically an outbreak of disease — to characterize it accurately, to define its extent and to identify its source or origin or the particular combination of host, environmental and agent factors which were responsible for its genesis? Or is he prepared ipso facto as a D.V.M. to determine in modern scientifically acceptable terms the level of endemicity of a widespread infection in an animal population in order to provide mortality and morbidity baseline data as a yardstick against which the progress of a future disease control program may be measured? Or, is he able to scientifically design a program for disease control or design and carry out a program of evaluation and measurement of disease control progress? Is he conversant enough with past efforts to be able to visualize new approaches to old or new problems? In sum, is he specifically trained for his job? Has he mastered and can he effectively use the epidemiological, statistical, immunodiagnostic and other basic tools which have evolved in recent years in population medicine and which must be successfully and innovatively applied if continued progress — and hopefully accelerating progress — is to be made in preventive veterinary medicine?

If a young veterinarian possessing only the DVM degree is sometimes not fully qualified to undertake tasks such as these, he is probably not as useful to the governmental organization to which he belongs as he might be. In addition, he personally finds more often than not that his activities become increasingly — and sometimes almost exclusively — the performance of more or less routine tasks laid down by others and that a creative and innovative, and hence highly rewarding role for him in the disease investigation and control process is lacking.

Experienced administrators of livestock disease control activities in government have long recognized the obvious limitations of the usual types of “on-the-job” training of a tutorial or short-course character in a specialty which is as dynamic and potentially challenging and exciting as is preventive (or, if you prefer, regulatory) veterinary medicine. For a number of years they have asked those of us in academic medicine for assistance in the planning of specialized graduate programs in our field. This is clearly an area in which we on the faculties of veterinary medicine have for too long neglected our responsibilities to both society and the profession. It should be a sign of some encouragement to all of us, therefore, that after so many years of unavailing insistence on the part of forward-thinking veterinarians in government, that the schools of veterinary medicine at long last have begun to respond to these most important needs of our profession for graduate training in epidemiology, biostatistics and other basic preventive medical disciplines.

The main purpose of my meeting with you this morning is to describe one
on-going graduate program which has been designed specifically to fulfill these needs. I refer to the graduate program for the professional degree of Master of Preventive Veterinary Medicine which has evolved over the last 3-4 years at the University of California. From the onset of planning, the design of this program has involved the cooperative efforts of senior veterinarians in the Animal Health Division of the USDA, as well as those in the California State Division of Animal Industry and our university faculty. During the two years that this program has been in operation we have ended each year with a full day's critique in which senior veterinarians from the federal and state governments, students currently in the program, and faculty have all freely and frankly participated. This has been such a valuable experience for all of us that we intend to make it a continuing feature of the program. In this way we are able to be immediately sensitive to new needs and flexible in their implementation.

The University of California has now graduated two MPVM classes totalling 19 veterinarians and a third class of 12 graduate students is currently enrolled. The USDA Animal Health Division sends five carefully selected veterinarians to us each year for the MPVM degree. This year we also have veterinarians from the state governments of California and Georgia, from the Veterinary Biologics Division of the ARS, from the USDA Consumer and Marketing Service, and from governmental and academic posts in Canada, Brazil and Peru. In fact, some foreign students have been sent to us each year by the World Health Organization, which has been actively interested in the MPVM program since its inception. In addition, in the past two years we have also had in the program one or two veterinarians directly from private practice or other backgrounds. In total these students bring with them a good mix of backgrounds and varied experiences. We intend to keep our classes that way.

I mentioned at the start, that the MPVM is a professional degree. I would like to explain that. At the time the Board of Regents approved our plans and program for this totally new veterinary degree, they amended their by-laws with respect to the School to state that the School of Veterinary Medicine of the University of California now offers two professional degrees, the Doctor of Veterinary Medicine and the Master of Preventive Veterinary Medicine, as well as the usual M.S. and Ph.D. research degrees. This amendment is important, because it is our aim and that of the governmental agencies who have worked with us in the development of this program that the MPVM eventually be accorded professional recognition by civil service, as well as by the agencies which employ veterinarians in the field of preventive veterinary medicine. We feel that such recognition of advanced degrees — as well as of specialty board credentials — is one important way both to accelerate staffing improvements and to enhance professional prestige in this field.

You are probably interested in knowing just how this program is organized. First of all, the MPVM program — except for its subject orientation — is roughly analogous to that for a Master of Public Health degree with concentration in epidemiology and biostatistics. That is, it is an 11 month program and all of its courses are conducted on a seminar or small group discussion basis. Although there are a number of required courses, there is sufficient flexibility in the program to permit some degree of specialization.

Thus, some individual students have concentrated their elective efforts in areas such as immunology, computer science, ecology, poultry diseases, food science and
virology, among others. In addition to this advanced course work, students, either individually or in groups of two, undertake an epidemiological research problem of their own choosing. While the main intent of this portion of the training is the application under supervision of tools which can be used in the field, I am pleased to say that several of these projects have yielded very useful publishable results. For instance, two students — partially through the systematic traceback of infected animals from slaughterhouses — have helped identify the existence and wide distribution in California of hydatid disease, a previously unreported zoonosis which is potentially of major economic and public health importance. Another student has utilized the Market Cattle Testing program and the University’s computer to carryout a detailed prevalence survey for anaplasmosis in California. Yet another student has done an extensive statistical study using some rather exciting new methods to help identify sources of animal losses in feedlots in southern California.

That in a nutshell is the MPVM program, one which we hope will before too long lead to similar programs in other veterinary schools.
CONTINUING EDUCATION
IN REGULATORY VETERINARY MEDICINE

The following advanced training is available to Federal and State veterinarians as part of continuing education in regulatory veterinary medical practice:

BRUCELLOSIS EPIDEMIOLOGY —
ADVANCED TWO YEAR PART-TIME POST GRADUATE STUDY

Conducted by the staff at the veterinary school, University of Minnesota. Subjects include advanced microbiology, serology, and related instruction to provide the capability to conduct a problem herd program in a State.

EPIDEMIOLOGY — PROGRAM IN PREVENTIVE VETERINARY MEDICINE
One Year

The program in preventive veterinary medicine has been developed by the University of California at Davis to provide Federal and State regulatory veterinarians with special knowledge and competence in epidemiology, biostatistics, mass disease prevention, control and eradication. Special disciplines include information retrieval, analysis and medical biostatistics. The degree of Master of Preventive Veterinary Medicine is awarded to successful candidates completing the program.

HOG CHOLERA —
EXTENDED EPIDEMIOLOGICAL AND DIAGNOSTIC TRAINING —
TWO TO TWO AND ONE-HALF YEARS FULL-TIME POST GRADUATE STUDY

A university study program for veterinarians on a continuing basis. The course is conducted by staffs of selected universities and includes epidemiology, immunology, bacteriology, virology, pathology, and biostatistics.

POULTRY DISEASES — POST GRADUATE PROGRAMS IN AVIAN MEDICINE AT SELECTED UNIVERSITIES — TWO OR MORE CALENDAR YEARS

Post graduate level programs for selected ANH veterinarians prepare candidates to deal with current or expected program problems. Selectees are nominated by the Veterinarian in Charge, recommended by the Poultry Diseases Staff, and selected by Assistant Directors. In addition, selectees must satisfy entrance requirements of the university involved.

TUBERCULOSIS —
EXTENDED EPIDEMIOLOGICAL POST GRADUATE TRAINING
Two Years

A university study program requiring half time spent in graduate work and half time spent in problem herd activities. The ANH Division cooperates with selected
universities which provide extended laboratory training with related courses in bacteriology, immunology, serology, and statistics.

ADDITIONAL GRADUATE SUBJECTS AVAILABLE TO REGULATORY VETERINARIANS:

- Veterinary Microbiology
- Veterinary Virology
- Immunology
- Pathology
- Neuropathology
- Biostatistics
- Epidemiology

UNIVERSITIES WHERE THE ABOVE GRADUATE SUBJECTS ARE AVAILABLE:

(Instruction not limited to these universities)

- University of California
- Iowa State University
- Johns Hopkins University
- University of Maryland
- University of Minnesota
- University of Missouri
- University of Nebraska
- University of Pennsylvania
- Texas A&M University

BRUCELLOSIS EPIDEMIOLOGISTS SEMINAR

40 Hours

Presented to a class of 60 brucellosis epidemiologists and conducted at selected locations by the ANH Division Brucellosis Staff and outside resource people. Current developments in brucellosis are reviewed so that a high level of competence may be maintained among previously trained brucellosis epidemiologists.

BRUCELLOSIS – SPECIALIZED TRAINING

80 Hours

Presented to a class of 12-15 veterinarians. The course is conducted by the Brucellosis Staff and outside resource people at selected locations. Practical training in brucellosis problem herd procedures is provided the participants.

EPIDEMIOLOGICAL ORIENTATION FOR VETERINARIANS

32 Hours

Presented to a class of 40-45 veterinarians on a regional basis. Subjects include the basic principles of epidemiology, basic biostatistical methods, and epidemiological problems related to diseases of livestock and poultry.
CONTINUING EDUCATION

EPIDEMIOLOGY – ADVANCED GENERAL

Prerequisite: completion of the basic epidemiological orientation for veterinarians course. The advanced epidemiology course presents some of the methods used in the application of epidemiology to the prevention, control and eradication of animal diseases. Subjects include a review of epidemiological and biostatistical principles, obtaining histories, disease investigation, animal demography, epidemiologic aspects of immunology-microbiology, ecological relationships, use of bibliographic tools in biomedical sciences and considerations of immunodiagnostic and other techniques for diagnosis of disease as applied to animal disease programs.

EQUINE DISEASES DIAGNOSTIC COURSE

Presented to a class of 30 veterinarians at selected locations. The course consists of lectures on influenza, arthritis, infectious anemia, piroplasmosis and leptospirosis in horses; a review of equine hematology followed by case studies and laboratory sessions in necropsies of horses infected with piroplasmosis and infectious anemia for pathological changes.

FOREIGN ANIMAL DISEASE DIAGNOSTIC TRAINING COURSE

Presented to 20 Federal veterinarians at the National Animal Disease Laboratory in Ames, Iowa, and Grose Isle, Quebec, Canada, by ANH Division staff and outside resource people. The course is designed to improve understanding of clinical symptoms and gross pathology of foreign animal diseases and their comparison with similar diseases. This course has not been offered to State employed veterinarians as diagnosis of foreign animal diseases is the responsibility of the Federal veterinarian.

FOREIGN ANIMAL DISEASE WORKSHOP

Presented to 20 veterinarians at the National Animal Disease Laboratory at ANH Division staff and outside resource people. The workshop provides trained diagnosticians an opportunity to review diagnostic capabilities with emphasis on the differential diagnosis of foreign and domestic animal diseases and to discuss unusual field cases investigated and the problems encountered in differential diagnosis.

HOG CHOLERA DIAGNOSTIC TRAINING COURSE

Presented to 15 veterinarians at the National Animal Disease Laboratory by the ANH Division Swine Diseases Staff, NADL Staff, and outside resource people, and the Animal Disease and Parasite Research Staff. The course consists of orientation in the principles of virology and immunology and the relationship of these
principles to hog cholera. The virus of hog cholera is studied, clinical symptoms, pathology (gross and microscopic), pathogenesis, and differential diagnosis with lectures and practical experience.

HOG CHOLERA DIAGNOSTICIANS SEMINAR

40 Hours

Presented to 30 veterinarians at the National Animal Disease Laboratory by staffs of the ANH, NADL, Veterinary Biologics, Animal Disease and Parasite Research Divisions and outside resource people. Completion of the Hog Cholera Diagnostic Training Course is prerequisite to attend the Seminar.

IMPORT-EXPORT ORIENTATION AND TRAINING CONFERENCE FOR VETERINARIANS IN CHARGE AND PORT VETERINARIANS

28 Hours

Presented to 25 veterinarians, including Veterinarians in Charge, Assistants, and port veterinarians. The conference consists of lectures, discussions, and workshops relative to importation and exportation of animals and animal products. The course is designed to include administrative functions, port activities, export health requirements, public relations, orientation by meat inspection services and background information on the responsibilities and activities of the Import-Export Headquarters Staff. The course will be conducted in Hyattsville or other locations as announced, by Division personnel and outside resource speakers.

LAWS, REGULATIONS, POLICIES

28 Hours

Presented to a class of 50-75 veterinarians and conducted on a regional basis. The course includes basic authorities under which the ANH Division operates, the implementation of these authorities by regulations, enforcement procedures, and policies and related laws administered by other government agencies. The Disease Control Services Staff, other ANH Division Staffs, and Agency speakers will serve as instructors.

LABORATORY ANIMALS TRAINING COURSE

35 Hours

Presented to 20 veterinarians and livestock inspectors at selected locations in the Northeastern, Southeastern, North Central and Western Regions. The course is conducted by the Laboratory Animals Staff and outside resource speakers. Training consists of procedures for conducting the necessary inspections; familiarity and uniform interpretation of regulations and standards; investigation and preparation of alleged violation cases; and understanding the scope of the Division’s responsibilities and contact with related special interest groups.
CONTINUING EDUCATION

POULTRY DISEASES – BASIC COURSE

64 Hours

Conducted on an annual basis by the ANH Division for approximately 16 veterinarians in disease control and supervisory positions in the ANH and cooperating State agencies. Instruction is provided by ANH Division personnel and outside resource specialists at the NADL. The course is designed to provide training for regulatory field veterinarians in basic information on avian anatomy, nutrition, physiology, pathology, serology, immunology, epidemiology, and their application in the prevention and treatment of domestic and foreign diseases of poultry. Industry and university veterinarians will be considered according to available space.

POULTRY DISEASES – ADVANCED COURSE

32 Hours

Presented to a class of 10 or more experienced State and Federal poultry disease epidemiologists. The course is designed as a survey of the most current research, service, and epidemiological developments in pathology, microbiology and population medicine, as well as in the prevention and treatment of domestic and foreign diseases of poultry. Refinements in the application of mathematical models to disease are reviewed. The course is conducted by ANH Division personnel and outside resource speakers. Industry and university veterinarians will be considered according to available space.

POULTRY DISEASES – SALMONELLA SEMINAR

35 Hours

Seminars for experienced State and Federal field and laboratory personnel related to the Salmonella program. Conducted for approximately 15 State and regional Salmonella epidemiologists and laboratory personnel. Seminars deal with current developments in program diagnosis and control.

POULTRY DISEASES – SALMONELLA DIAGNOSTIC TECHNIQUES

36 Hours

Designed for ANH, State, and industry employees who are engaged in laboratory diagnosis of animal diseases. The course consists of basic laboratory procedures for isolation and identification of Salmonella. Emphasis is placed upon isolation of Salmonella from specimens of animal tissues and samples of feed and feed ingredients.

POULTRY DISEASES – ADVANCED SALMONELLA DIAGNOSTIC COURSE (GROUPINGS)

36 Hours

Students are instructed in the techniques used in preliminary Salmonella serology. The procedures for “O” antigen grouping and preliminary identification
of "H" antigens by the Spicer-Edwards technique are taught. The course is designed for ANH, State, and industry laboratory personnel.

POULTRY DISEASES – MYCOPLASMA DIAGNOSTIC LABORATORY TECHNIQUES

12 Hours

One and one-half days of lecture and discussion in which serological procedures are demonstrated for mycoplasma diagnostic techniques. Students actually conduct the tests for mycoplasma identification. ANH, State, and industry personnel are selected for this course.

PROFESSIONAL DEVELOPMENT SEMINAR FOR VETERINARIANS

28 Hours

Presented to a class of 25 veterinarians with priority given to those more recently employed. The course is conducted on a regional basis. The object of the Seminar is to provide basic and current information concerning cooperative programs, define the scope of Division responsibilities, the State-Federal cooperative relationship, line, staff, and administrative functions in carrying out programs, and career opportunities.

RADIOLOGICAL MONITORING

20 Hours

Presented to groups of 20 State and Federal veterinarians and inspectors. It is conducted by ANH and other USDA personnel at various locations. The training provides a capability in the use of radiological monitoring instruments and protection against fallout and other dangers to livestock and people through nuclear attack.

RADIOLOGICAL MONITORING FOR INSTRUCTORS

40 Hours

The training is presented to groups of approximately 25 employees and conducted at various State universities and by the Department of Defense at Battle Creek, Michigan. Apart from a capability to serve as an instructor in radiological monitoring, employees learn to assess fallout damage and dangers to livestock and people through nuclear attack.

RADIOLOGICAL DEFENSE OFFICER TRAINING

40 Hours

This training is presented to groups of 25 employees who have received instructor training. Advanced training in the calculation of fallout, assessment of damage and in the duties of the Radiological Defense Officer as a member of State and Defense Boards are included in the instruction.
CONTINUING EDUCATION

CBR WEAPONS ORIENTATION COURSE

Top secret security clearance is required for admission. The mission of the course is to prepare and present material dealing with United States doctrine techniques and capabilities in the field of Chemical Biological and Radiological operations as an orientation for senior Department of Defense military and civilian personnel and selected personnel of other governmental departments.

SCABIES TRAINING COURSE

Presented to groups of 20 Federal and State regulatory personnel engaged in animal disease eradication programs. The course is conducted by ANH personnel of the Technical Services Staff. It provides the capability of recognizing scabies suspicious animals, collecting specimens, identifying infected animals and handling pesticides. The course is conducted at the NADL and in Beltsville, Maryland.

SCREWORM-BLOWFLY IDENTIFICATION COURSE

Presented to groups of 12 State, Federal and outside personnel engaged in the Screwworm Eradication Program activities and in the inspection of livestock for disease. It is conducted by the ANH Division personnel of the Technical Services Staff and/or the Mission, Texas, facility staff. Instruction includes the characteristics of the Screwworm fly and larvae as compared to the Blowfly. Identification of the Screwworm Fly is essential to an effective eradication program. The course is conducted in Beltsville, Texas, or Laredo, Texas.

TICK IDENTIFICATION COURSE

Presented to groups of 10 employees engaged in the inspection of livestock such as in the tick eradication program. It is conducted by personnel of the ANH Division’s Technical Services Staff and instruction includes providing a capability to distinguish the Texas Fever Tick from other species which resemble the Texas Fever Tick and are of varying importance in disease eradication. The course is conducted in Laredo, Texas and Beltsville, Maryland.

TUBERCULOSIS – POST MORTEM EXAMINATION OF REACTORS

Available to ANH field veterinarians through the Livestock Slaughter Inspection Division of the Consumer and Marketing Service, at Fort Worth, Texas; Los Angeles, California; Omaha, Nebraska; and St. Paul, Minnesota. One or two veterinarians receive training at the same time. The training familiarizes the veterinarian with post-mortem procedures for the inspection of cattle that are tuberculosis reactors.
The course conducted at the National Animal Disease Laboratory consists of 12 hours of laboratory demonstrations which include histopathological diagnosis of granulomatous lesions, culture and typing of the mycobacteria and post-mortem of tuberculous laboratory animals. The remaining 16 hours of lecture and discussion cover interpretations of laboratory reports and field implications of the various findings as well as other epidemiological aspects of tuberculosis eradication.

**VETERINARY ADMINISTRATOR DEVELOPMENT PROGRAM**

10 Months

The Veterinary Administrator Development Program provides replacements for important administrator positions. A minimum of five years experience in veterinary medicine is required for nomination for the program. Three of the five years should be with the ANH Division. Veterinarians in Charge nominate candidates, approval of the Assistant Director is required, and the Personnel Committee recommends selected candidates to the Director for approval.

**MANAGERIAL GRID – PHASE I**

60 Hours

Presented to a class of approximately 50 Federal and State field, administrative and administrator personnel. The course consists of theories showing how concern for people and concern for production can be integrated within an organization to increase output and reduce costs. Ways are studies to pool resources when more than one person is involved in getting the job done and for using the GRID theories to diagnose problems preventing effectiveness of the organization.

**FIRST LEVEL SUPERVISOR COURSE**

40 Hours

Presented to a class of approximately 25 Federal and State employees who have assumed or will assume supervisory responsibilities in the near future. Subject matter includes personnel policies, practices, procedures, supervisory responsibilities within the organization, communicative processes in management, human behavior, motivation, work planning and productivity. The course is offered on a regional basis throughout the country.

**MODERN SUPERVISORY PRACTICE**

30 Hours

A correspondence course available through the USDA Graduate School in Washington, D.C. Tuition and fees amount to $71.00. Lessons cover subjects of major importance to supervisors including communications, motivation, supervisory principles and techniques, training, participation, work improvement, planning, organizing and scheduling.
SEMINAR IN EXECUTIVE DEVELOPMENT

40 Hours

Tuition $200.00 plus per diem. Courses are conducted in Front Royal, Virginia; San Francisco, California; and Kansas City, Missouri.

The program is designed to broaden executive thinking. The program content explores three levels of agricultural involvement — the world community of nations, the national community of special interests, and the community of USDA agencies. Participants from Federal and State agencies attend the program.

EXECUTIVE SEMINARS — U.S. CIVIL SERVICE COMMISSION
Kings Point, New York and Berkeley, California
Tuition $475.00 — Per Diem is $2.50 per day

80 Hours

The objective of the Executive Seminar is to broaden the conceptual understanding and enhance the administrative abilities of mid-level executives. Courses include Administration of Public Policy, Environment of Federal Operations, Federal Program Management and Management of Organizations.

LEGISLATIVE OPERATION ROUNDTABLE FOR EXECUTIVES
U.S. CIVIL SERVICE COMMISSION - Tuition $130.00

40 Hours

This program is co-sponsored by the U.S. Civil Service Commission and American Political Science Association and designed to give Federal career executives increased knowledge of congressional activities. Topics include powers, functions and organization of Congress, the legislative process, politics and congressional action, internal-external pressures on Congress, policy formation, legislative-executive relationships, and congressional review.

STATE GOVERNMENT AND ADMINISTRATION PROBLEMS,
PROGRAMS, PLANNING

40 Hours

U.S. Civil Service Commission conducts the course in Washington, D.C. for Federal and State agency employees. The cost is $180.00 (per diem additional). The course is designed for officials with responsibilities requiring a broad understanding of the intergovernmental relations framework and also for those with assignments relating specifically to State Programs.

For additional information about any of the Educational Opportunities listed contact your State Veterinarian, Federal Veterinarian in charge or write directly to Dr. E. E. Saulmon, Director, Animal Health Division, USDA ARS, Federal Center Building, Hyattsville, Mo.
REPORT OF THE COMMITTEE ON
PROFESSIONAL EDUCATION AND EXTENSION
USAHA – 1969

Samuel Guss, University Park, Pa., Chairman; Clifford Beck, East Lansing, Mich.;
George L. Crenshaw, Davis, Calif.; C. Dobbins, Athens, Ga.; S. H. Flora,
Lincoln, Nebr.; H. G. Geyer, Washington, D.C.; R. C. Hammond, College Park,
Hostettler, Pullman Wash.; Moses Simmons, Denton, Tex.; T. P. Siburt,

The Committee on Professional Education and Extension met yesterday
afternoon in the Detroit Room of this hotel. Actions resulting from Committee are
as follow:

1. The Committee recommends that the Executive Secretary of this
   Association write to the Director of the National Agriculture Library to
   ask that proceedings from the meetings of this Association be indexed by
   the library in such a way that governmental agencies and others who need
   this important reference material can find it easily. It is a fact that much
   valuable material in our annual proceedings books is virtually inaccessible
   to those who cannot remember when such material was presented or to
   those who can not take the necessary hours of diligent searching to find it.
   We are thinking in terms of five year accumulative indices.

2. The Committee respectfully requests the Executive Committee to ask all
   of the appropriate agencies of government and the working committees of
   this association to inform The Federal Extension Service of actions,
   regulations and program developments of interest to this Association. This
   information can be quickly relayed to states regulatory officials and
   extension veterinarians. We feel that this will close a serious communica-
   tions gap which has not kept those who need to know informed quickly
   enough. Members of the Committee revealed a number of recent instances
   when the objectives of this Association were deferred by too slow
   communication. We strongly urge that effort be made to develop this
   communication channel as soon as possible.

3. This Committee is also happy to report that the Agricultural Board of the
   National Research Council has begun a project to develop a National
   Animal Disease Morbidity and Mortality Reporting System. The Board
   originally proposed an interdisciplinary study period followed by a pilot
   project in which a recommended plan could be studied during a trial
   period. An interdisciplinary team consisting of two biometricians, a
   laboratory diagnostician, an economist and a veterinary epidemiologist is
   expected to submit a plan to meet the Board’s comprehensive objectives
   within a few weeks. The federal government has provided the money and
   the personnel to implement what is being done. It is regrettable that
   solicited funds from sources other than government agencies have not
   materialized. However, there may be a need for all the support this
   association can muster to implement a pilot program when a proposal is
   submitted by the interdisciplinary team.

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4. In connection with the foregoing problem, this committee unanimously endorses reports by other committees of this association to bring into existence a uniform national system for animal identification. We strongly urge the Executive Committee of this association to make the moves it deems most appropriate to implement such a system as soon as possible.

5. Responding to a request from President O'Hara, our Committee will prepare for the current proceedings of this meeting, a list of continuing professional education programs. This list will be designed primarily for use by regulatory veterinarians who want to improve their competence in subject matter areas related to their work. This list will be prepared in consultation with the AVMA Continuing Education Director and the U.S. Department of Agriculture and Health, Education and Welfare. This will not entail a lengthy description of graduate programs, but rather a listing of programs offered and persons to contact about them.

Mr. Chairman, these recommendations and decisions constitute the actions of the Committee on Professional Education and Extension and they are respectfully submitted.
STATUS OF THE STATE-FEDERAL SALMONELLA PROGRAM

by

Saul T. Wilson, Jr., D.V.M., M.P.H.*
John W. Walker, D.V.M.**
Claude J. Pfow, D.V.M.***

Presented at the
Seventy-third Annual Meeting
United States Animal Health Association
Milwaukee, Wisconsin
October 12-17, 1969

The list of diseases receiving the attention of Cooperative State-Federal Animal Health Officials was extended in 1967 to include Salmonella. I have the honor today of presenting the first report of the status of the Cooperative State-Federal Salmonella Program to a general session of this Association.

This program reflects the response of Animal Health Officials to the emergence of bacteria of the genus Salmonella as modern day disease agents of both public health significance and economic importance. The position Salmonella occupies today is one indication of the success achieved in efforts to eradicate those diseases of livestock that were of major public health importance at the turn of the century. Salmonellosis is of major concern to exporters of American livestock and poultry products, to the feed processing industry, and to domestic processors of our livestock and poultry or their products. Some countries who import American livestock and poultry have requested salmonellosis be included on the export health certificate in the certification regarding diseases. Chicken and turkey breeders are receiving an increased number of requests for hatching eggs that originate from flocks negative to tests for Salmonella, and for delivery of day old poults and chicks free of Salmonella. It is our personal opinion that in the future such requirements will become more stringent rather than remain the same or become less.

During the past year a Committee of the National Academy of Sciences National Research Council completed an examination of the Salmonella problem in the United States.1 Their primary concern was with human health. In their report the estimated incidence of human salmonellosis is placed at about two million cases annually and the total annual cost to the American economy at not less than 300 million dollars.

This Committee of the National Academy of Sciences recognized the infeasibility of total eradication of salmonellosis at this time but felt a great deal of

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improvements could be realized through minimizing infections among domestic animals, control of contamination of feed and feed ingredients, better food handling practices, protection of processed foods, improved sanitary practices in commercial kitchens, hospital wards, and the food preparation area of the home. You will note many of these areas are beyond the scope of responsibilities of Animal Health Officials.

In considering the relationship of Salmonella infections in livestock and poultry to human health, the concern is not only with the clinically ill animal but of probably more importance, the apparently healthy carrier animal.

While we have developed considerable expertise in dealing with the tuberculosis lesion animal that fails to react to the tuberculin test, and the non-reactor brucella shedder, we have seldom, if ever, encountered these animals on a herd basis. Salmonella infections are most often subclinical and available information indicates they exist on a herd or flock basis. These are flocks and herds of apparently healthy livestock in which no evidence of disease exists. Developing effective control measures for application at the production level offers a tremendous challenge to our profession. It opens up a new area for exploration by regulatory veterinarians.

Current program activities are directed toward a reduction in the level of Salmonella contamination in protein supplements of animal and marine origin used in livestock and poultry feeds. The program is voluntary and involves the State agency that has regulatory responsibility for preventing the spread of diseases through animal feed and feed ingredients, the rendering industry, the industrial fish industry, and the Animal Health Division (ANH), U.S. Department of Agriculture. The program became national in scope with all fifty States and Puerto Rico participating when South Dakota, Nebraska and Puerto Rico entered the program during Fiscal Year 1969.

The Salmonella Uniform Methods and Rules \( ^2 \) (SUMR) recommended by this Association at the 1968 meeting have been adopted by the Animal Health Division, U.S. Department of Agriculture, as satisfactory standards for the conduct of cooperative program activities. A number of States who had programs for the control of Salmonella in animal feeds prior to the initiation of the State-Federal program have modified their sampling procedures to make them equivalent to or more stringent than the standards of the Uniform Methods and Rules. The SUMR calls for the program to go through three phases on an individual plant basis; Evaluation, Clean-Up and Approved. During the evaluation phase of the program the Salmonella status (positive or negative according to program procedures) of the finished product of each plant is determined. In the clean-up phase studies are initiated to identify sources of product contamination. During this phase management will develop a plant Salmonella program and apply procedures to eliminate sources and potential sources for contamination. In the approved phase, plant management will carry out the Salmonella control program developed in the clean-up phase for their plant. The activities of State and Federal inspectors in approved plants will serve to verify the adequacy of the plant's control program. Major program activities in Fiscal Year 1969 evolved around the evaluation of plants.

The following statistics summarizes these activities. The information regarding plant evaluation and classification is based upon replies to a questionnaire sent to the ANH Veterinarians in Charge in August of this year.
Salmonella Program

FIGURE 1

STATES COMPLETING EVALUATION OF ALL PLANTS
As of June 30, 1969

FIGURE 2

Salmonella Program

EVALUATION OF PLANTS

EVALUATED 30%
(286 Plants)

22%

70%

22%

EVALUATED 78%
(744 Plants)

NOT EVAL.

NOT EVALUATED

June 30, 1968

June 30, 1969

TOTAL PLANTS: 950
There were 950 plants in the United States and Puerto Rico that processed animal or marine proteins for use in animal feeds during Fiscal Year 1969. At the close of the year 80% or 678 of the 845 rendering plants had been evaluated. While 63% or 66 of the 105 marine plants had been evaluated. The difference in the proportion of marine and rendering plants evaluated may be attributed to the seasonal operations of the marine plants.

The number of plants in each stage of evaluation is given in Table 1. There were 29 plants that had not received an initial inspection at the close of the fiscal year, of which 13 are packer renderers located in one State.

<table>
<thead>
<tr>
<th>EVALUATION INSPECTIONS</th>
<th>NUMBER AND TYPE OF PLANTS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed</td>
<td>Required</td>
<td>Marine</td>
</tr>
<tr>
<td>None</td>
<td>Three</td>
<td>8</td>
</tr>
<tr>
<td>One</td>
<td>Two</td>
<td>12</td>
</tr>
<tr>
<td>Two</td>
<td>One</td>
<td>19</td>
</tr>
<tr>
<td>Three</td>
<td>None</td>
<td>66</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>Three</td>
<td>105</td>
</tr>
</tbody>
</table>

*Includes inspections made in Fiscal Year 1968.
**Includes plants inspected but have ceased operations since initiation of program.

This State did not include packer renderers in their program at the beginning of the year. The twenty-six States that have completed the evaluation of all their plants are indicated in Figure 1. The number of plants in these States varied from one in Vermont and West Virginia to 51 in Iowa.

Progress in completing the evaluation phase of the program is shown in Figure 2. At the close of Fiscal Year 1968, 31% (268) of the 950 plants had been evaluated (according to the Salmonella Uniform Methods and Rules) as compared to 78% (744) of the 950 plants at the close of Fiscal Year 1969. The number and percent of plants evaluated in Fiscal Year 1968 and 1969 that were classified as negative is presented in Table 2. While the number of plants evaluated in 1969 was 60% greater than the number of plants evaluated in 1968, the proportion of plants meeting the criteria of the Salmonella Uniform Methods and Rules for a negative classification were similar, 26% in 1968 and 30% in 1969.
TABLE 2 – NUMBER OF PLANTS EVALUATED BY TYPE, NUMBER AND PERCENT NEGATIVE* BY YEAR OF EVALUATION

<table>
<thead>
<tr>
<th>TYPE</th>
<th>EVALUATED IN FY 68</th>
<th>EVALUATED IN FY 69</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Plants</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>MARINE</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>RENDRINGG</td>
<td>270</td>
<td>66</td>
</tr>
<tr>
<td>TOTAL</td>
<td>286</td>
<td>74</td>
</tr>
</tbody>
</table>

*AAccording to criteria of the Salmonella Uniform Methods and Rules. A negative plant is one that has had three consecutive negative tests of 10 sample units (total 30 negative sample units) of an official sample of finished product. The sample units are collected from the shipping or storage area at an interval of not less than 30 days apart within a 12 month period.

A comparison of the status of the 286 plants evaluated in 1968 at the close of Fiscal Year 1969 is presented in Table 3. It was encouraging to note that the number of negative plants in this group increased from 74 to 89 plants.

TABLE 3 – COMPARISON OF THE STATUS OF 286 PLANTS EVALUATED DURING FISCAL YEAR 1968 AT CLOSE OF FISCAL YEAR 1969

<table>
<thead>
<tr>
<th>PLANTS</th>
<th>STATUS 1968</th>
<th>STATUS 1969</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TYPE</td>
<td>NUMBER</td>
</tr>
<tr>
<td>MARINE</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>RENDRINGG</td>
<td>270</td>
<td>66</td>
</tr>
<tr>
<td>TOTAL</td>
<td>286</td>
<td>74</td>
</tr>
</tbody>
</table>

Field stations reported the examination of 30,645 samples for salmonellae of which 5,188 were positive. Finished product accounted for 22,824 of the samples with 3,835 (16.8%) being positive. Inline-product and environmental samples accounted for the other 7,821 samples with 1,353 (17.3%) being positive. The number of finished product samples tested and the number of percent positive by plant category is presented in Table 4.
Forty percent of the samples collected in plants classified as blenders were positive. This is more than twice the percentage of positive samples obtained in either of the other categories of plants processing animal proteins. This is an area that should receive increased attention if we expect to effect a reduction in contamination of animal byproducts going into finished feed. Special attention was called to this fact in the 1969 Report of the Salmonellosis Committee. It should also be noted that the proportion of positive samples obtained in plants associated with the slaughter of poultry did not differ from the proportion of positive samples obtained in plants associated with the slaughter of livestock. The proportion of positive samples obtained in plants processing marine products remains lower than that for plants processing animal byproducts.

The results of the statistical methods employed in analyzing the data in Table 4 are summarized below.

1. The percent of positives for plants represented in the blending category is significantly different from each of the other categories at the 0.01 significance level.

2. The percent of positives for livestock slaughter plants is significantly different from plants in the marine products and feathermeal category at

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*99% confidence intervals and chi-square tests where there were overlaps between plant categories in the confidence intervals for the percent positives.
the 0.01 significance level and from plants in the independent category at the 0.05 significance level. However, the magnitude of the difference between livestock slaughter and independents is small and could be due to other reasons than type of plant, since variance within categories is ignored.

3. The percent of positives for poultry slaughter plants is significantly different from marine products and feathermeal plants at the 0.01 significance level. It is not significantly different from plants in the independent category or plants in the livestock slaughter category.

4. The percent of positives for plants represented in the independent category is significantly different from plants in the marine products and feathermeal category at the 0.01 significance level.

5. The percent of positives for plants in the marine products category is significantly different from plants in the feathermeal category at the 0.01 significance level.

The results of serotyping 3,996 Salmonella isolates from samples collected in marine product and rendering plants were reported on ANH Form 9-10. These serotypes are listed in descending order of frequency in Appendix I and in alphabetical order in Appendix II of this report. The number of isolates differs from the total number of positive samples because all isolates were not submitted for serotyping. There were 93 different serotypes represented in these isolates, as compared to 72 different serotypes represented in the 2,175 Salmonella isolates serotyped for program purposes in Fiscal Year 1964. The five most frequent serotypes were *S. senftenberg* (9.4%), *S. montevideo* (9.13%), *S. eimsbuettel* (8.8%), *S. anatum* (6.9%) and *S. derby* (4.6%). Each of the 25 serotypes that occurred most frequently in man during 1963-67, as reported by the National Communicable Disease Center, is included in these isolates with the exception of Paratyphi B. and *S. typhi*. (Table A3, pages 185-186, NAS Report)

This program is based upon the hypothesis that the presence of Salmonella in a product that undergoes sufficient heat treatment for the destruction of Salmonella represents subsequent contamination. During the past year we have had an opportunity to observe the relationship of the results of tests for salmonellae to the sanitary practices within plants.

The information collected on the plant inspection report (ANH Form 9-9) permits a characterization of individual items or practices as they relate to sanitation but does not provide for an overall characterization of the plant.

To obtain data for correlating test results with sanitation in individual plants each field inspector was asked to indicate the sanitary condition of each plant he had evaluated and to categorize as either Very Good, Good, Fair, or Poor. The standards for categorizing were the Recommended Sanitation Guidelines. Data was received for 708 of the evaluated plants of which 65 were processors of marine products and 643 were processors of animal byproducts. Data was not received from one State containing 35 evaluated rendering plants and one evaluated marine product plant. The categorization represents the subjective judgment of the individual inspectors. A comparison of the sanitary condition as indicated by the field inspectors and the results of the laboratory examinations for Salmonella during plant evaluation is given in Table 5.
TABLE 5 — RELATIONSHIP BETWEEN SANITARY CONDITION AS REPORTED BY STATE AND FEDERAL FIELD INSPECTORS AND THE RESULTS OF LABORATORY EXAMINATIONS FOR SALMONELLA IN FINISHED PRODUCT SAMPLES OF PLANTS EVALUATED AT CLOSE OF FISCAL YEAR 1969.

<table>
<thead>
<tr>
<th>SANITARY CONDITION</th>
<th>MARINE PRODUCT PLANTS</th>
<th>RENDERING PLANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOTAL</td>
<td>NEUTRAL</td>
</tr>
<tr>
<td>VERY GOOD</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>GOOD</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>FAIR</td>
<td>31</td>
<td>22</td>
</tr>
<tr>
<td>POOR</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>65</td>
<td>37</td>
</tr>
</tbody>
</table>

Chi-square 3df=12.01, P < 0.01

*According to the Salmonella Uniform Methods and Rules a negative plant is one that has had three consecutive negative tests of 10 sample units (total 30 negative sample units) of an official sample of finished product. The sample units are collected from the shipping or storage area on three separate visits at an interval of not less than 30 days apart within a 12 month period.

The statistical tests for this data indicates the differences are due to a difference between poor plants and the combination of very good, good, and fair plants with very little difference among very good, good, and fair plants. In submitting this data to statistical tests we are evaluating a subjective rating system. Any differences that there may be in contamination rates among plants in each category and among evaluators is not accounted for in this analysis. No doubt there are other factors influencing contamination that have not been examined. These findings, however, are consistent with the hypothesis that good sanitary practices, as opposed to poor sanitary practices, may aid in lowering product contamination.

DISCUSSION

The task now before us is to complete the evaluation phase of the program in all plants and attempt to identify the sources of contamination in plants operating in compliance with the Sanitation Guidelines. This is a prerequisite for establishing realistic national goals regarding achieving approved status.

Phase II of the Salmonella Uniform Methods and Rules calls for us to work with individual plant managers in developing plant operated Salmonella control programs. These programs should be tailored to specific operations on an individual plant basis. They should be designed to prevent and minimize contamination of a plant’s finished product and to eliminate sources and potential sources for contamination within a plant.

A plant’s Salmonella control program is not complete if it does not include frequent product testing (at least every two weeks or more often if circumstances
indicate) in a plant operated or commercial laboratory.

Goals for plant improvements should be developed between inspectors and plant management tailored to specific operations on an individual plant basis. These goals should be a part of and not separate from other improvements scheduled for the plant. Plant managers should be informed of the specific standards they should strive to achieve in their plants.

Much of the present equipment in many plants cannot be easily cleaned. There is an urgent need for manufacturers of equipment for rendering plants to give increased attention to the design of equipment that can be easily cleaned and sanitized.

When existing equipment and facilities are replaced or remodeled, management should give special attention to eliminating those areas of contamination that can be controlled or eliminated through equipment design and structural arrangement of equipment and facilities.

The success of this program, as with all disease control efforts, depends upon active sustained interest, support and direction from the industries involved. The National Renderers Association, the National Fish and Oil Dealers Association, the American Feed Manufacturers Association, the Feed and Grain Dealers Association and representatives of the Car Service Committee of the American Association of Railroads, have all played an active role in meeting the responsibilities in developing practical procedures for the control of Salmonella transmission through feeds.

The coordination of this program's activities with the Food and Drug Administration and the Bureau of Commercial Fisheries has resulted in avoiding duplication of efforts between State and Federal agencies in the inspection of individual plants. Personnel of the National Communicable Disease Center assisted in the conduct of Salmonella Seminars for program personnel that were held in Minnesota and Alabama. State and Federal animal disease control personnel have provided assistance to State and Federal public health officials and to State and Federal meat inspection officials in the conduct of investigations related to salmonellosis as requested.

Traditionally disease control veterinarians have been concerned with the eradication of disease at the producer level. The elimination of Salmonella from animal feeds will reduce but not eliminate the total problem of salmonellosis in livestock. The degree of reduction is unknown. As fast as the industries involved in the production of animal feeds develop satisfactory in-plant Salmonella control programs our efforts should be shifted to assisting producers in the development and maintenance of flocks and herds free of the twelve Salmonella serotypes that have been responsible for over 70 percent of the cases in humans during the past ten years and the ten serotypes which are most often associated with morbidity and mortality losses in livestock and poultry. Five of these serotypes are common to both groups. An initial effort should be an accurate definition of the extent of the problem at the producer level. A practical procedure for a nationwide survey using a probability sample of poultry flocks for Salmonella has been demonstrated.7

Dr. Bendix stated in an address to the 1966 meeting of this Association, "The Salmonella problem is not one that will go away if we try to ignore it in our expanding and increasingly complex society."

"It is not an unsolvable problem. We have faced and overcome problems as complex as this one in the past. We have actually made responsible and effective
### APPENDIX I

**SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1969**

(Listed in Descending Order of Frequency)

<table>
<thead>
<tr>
<th>Salmonella</th>
<th>Number Isolates</th>
<th>Percent of Total Serotyped</th>
<th>Salmonella</th>
<th>Number Isolates</th>
<th>Percent of Total Serotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>senftenberg</td>
<td>378</td>
<td>9.46</td>
<td>minneapolis</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>montevideo</td>
<td>365</td>
<td>9.13</td>
<td>panama</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>eimbrettel</td>
<td>352</td>
<td>8.81</td>
<td>putten</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>anatum</td>
<td>275</td>
<td>6.88</td>
<td>urbana</td>
<td>7</td>
<td>0.17</td>
</tr>
<tr>
<td>derby</td>
<td>183</td>
<td>4.58</td>
<td>albany</td>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>infantis</td>
<td>157</td>
<td>3.93</td>
<td>braenderup</td>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>oranienburg</td>
<td>152</td>
<td>3.80</td>
<td>munchen</td>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>tennessee</td>
<td>147</td>
<td>3.68</td>
<td>tallahassee</td>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>bredeney</td>
<td>144</td>
<td>3.60</td>
<td>ohio</td>
<td>5</td>
<td>0.13</td>
</tr>
<tr>
<td>thomasville</td>
<td>131</td>
<td>3.28</td>
<td>halmsdad</td>
<td>4</td>
<td>0.10</td>
</tr>
<tr>
<td>cerro</td>
<td>115</td>
<td>2.88</td>
<td>san-diego</td>
<td>4</td>
<td>0.10</td>
</tr>
<tr>
<td>binza</td>
<td>112</td>
<td>2.80</td>
<td>Untypable-Group Q</td>
<td>4</td>
<td>0.10</td>
</tr>
<tr>
<td>Livingston</td>
<td>103</td>
<td>2.58</td>
<td>chester</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>bareilly</td>
<td>93</td>
<td>2.33</td>
<td>java</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>cubana</td>
<td>81</td>
<td>2.03</td>
<td>labadi</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>minnesota</td>
<td>79</td>
<td>1.98</td>
<td>motade</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>worthington</td>
<td>72</td>
<td>1.80</td>
<td>simbury</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>california</td>
<td>65</td>
<td>1.63</td>
<td>taksony</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>schwarzengrund</td>
<td>65</td>
<td>1.63</td>
<td>arkansas</td>
<td>2</td>
<td>0.05</td>
</tr>
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<td>62</td>
<td>1.55</td>
<td>bornum</td>
<td>2</td>
<td>0.05</td>
</tr>
<tr>
<td>drypool</td>
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<td>1.53</td>
<td>corvallis</td>
<td>2</td>
<td>0.05</td>
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<td>60</td>
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<td>dessau</td>
<td>2</td>
<td>0.05</td>
</tr>
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<td>56</td>
<td>1.40</td>
<td>madeia</td>
<td>2</td>
<td>0.05</td>
</tr>
<tr>
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<td>1.30</td>
<td>mihawashima</td>
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<td>0.05</td>
</tr>
<tr>
<td>typhimurium</td>
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<td>1.20</td>
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<td>0.05</td>
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<td>munster</td>
<td>38</td>
<td>0.95</td>
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<td>0.05</td>
</tr>
<tr>
<td>habana</td>
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<td>0.85</td>
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<td>0.05</td>
</tr>
<tr>
<td>alachua</td>
<td>32</td>
<td>0.80</td>
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</tr>
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<td>illinois</td>
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<td>0.03</td>
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<td>albert</td>
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<td>0.67</td>
<td>amager</td>
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<tr>
<td>amsterdam</td>
<td>26</td>
<td>0.65</td>
<td>berc</td>
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<td>0.03</td>
</tr>
<tr>
<td>meleagris</td>
<td>26</td>
<td>0.65</td>
<td>carmel</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>thompson</td>
<td>26</td>
<td>0.65</td>
<td>carrau</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>canoga</td>
<td>22</td>
<td>0.55</td>
<td>champagne</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>johannesburg</td>
<td>22</td>
<td>0.55</td>
<td>dublin</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>westhampton</td>
<td>28</td>
<td>0.45</td>
<td>jafa</td>
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</tr>
<tr>
<td>give</td>
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<td>0.37</td>
<td>jiviana</td>
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<td>0.03</td>
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<tr>
<td>newport</td>
<td>15</td>
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<td>kintambo</td>
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<td>grampensois</td>
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<td>lille</td>
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<td>manila</td>
<td>14</td>
<td>0.35</td>
<td>litchfield</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
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APPENDIX II
SALMONELLA SEROTYPES ISOLATED FROM
ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1969
(Listed in Alphabetical Order)

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beginnings with less information than we now possess about the salmonellae.” In my opinion we should have no fear for the problems that will be involved in assisting industry to develop flocks and herds free of the most frequently occurring Salmonella serotypes that are of economic or public health importance.
REFERENCES


ACKNOWLEDGEMENT

The authors wish to thank Dr. Victor C. Beal, Jr., Biometrician, Biometrical Services, ARS, USDA, for his assistance in the statistical analyses and interpretation mentioned in this report, his helpful suggestions are gratefully acknowledged.
REPORT OF SALMONELLA COMMITTEE

Chairman: A. A. Erdmann, Madison, Wis.

The Committee on Salmonellosis was established in 1966 and charged to be concerned with the scientific and professional aspects of the Salmonella problem. Prior to that time several committees of this association gave attention to different aspects of the problems related to Salmonella. These included the committees on Public Health and Radiological Fallout, Meat and Milk Hygiene and Transmissible Diseases of Poultry.

We have reviewed the original charge to this Committee and accomplishments to date. The major accomplishments have been in the adoption of uniform procedures and terminology for activities relating to Salmonella control. These have included uniform laboratory procedures for isolation of Salmonella, specified sampling procedure for use in the Salmonella program and a Model Rendering Plant Law.

In 1965 twenty-five states indicated having laws relating to rendering plants, many of which were vague and did not provide for adequate enforcement. Today 33 states have laws equivalent to the Model Rendering Plant law adopted by this association. Earlier this year the Council of State Governments gave their endorsement to this model law as published in 1967 Proceedings of this association (USAHA) and recommend it to the states for adoption where needed.

A training course for inspection personnel in the form of a Salmonella Seminar was held in St. Paul, Minnesota, for personnel of the North Central States and in Birmingham, Alabama, for personnel of the Southeastern States. A conference for personnel in the Western States and another for the Northeastern States is scheduled for fiscal year 1970.

This Committee urges the individual states to conduct a seminar or training session on Salmonella for local personnel as a sequel to the regional seminar.

A Government Industry Carrier Committee is currently developing recommended standards for railcars used for handling and transporting rendered animal by-products and fish meal. The assistance and attitude of the representatives of the American Association of Railroads has been most encouraging. Representatives of the American Feed Manufacturers Association, the Fish Meal and Oil Association, and the National Renderers Association have been cooperative in trying to arrive at a practical solution to this problem. In addition recommendations should be developed for other types of common carriers.

It is a common practice for blenders to purchase the output of several renderers and by mixing and blending prepare a finished product of a given protein and fat level for use by feed manufacturers. It is possible for negative rendered material from several establishments to be mixed with contaminated material from
a single producer and thereby contaminate the entire finished product of the blender. The Committee recommends a concentrated effort be made to encourage blenders to purchase rendered material from plants producing a negative product or to develop a process of terminal treating to insure a negative blended finished product.

The United States Department of Agriculture, Agricultural Research Service, Animal Health Division cooperative program with the states, coordinated with enforcement and compliance activities of the Food and Drug Administration, along with the educational and cooperative efforts of the National Renderers Association, Inc., the Fish Meal Importers Association, the National Fish Meal and Oil Association, and other industry groups has been commendable. These activities are encouraged and in light of the recommendations and conclusions of the eminent scientists comprising the National Academy of Science/National Research Council Salmonella Committee, it is urged that the tempo of their activities be increased and additional pressures exerted to bring about more rapid improvements in the industry, thus reducing the incidence of salmonellae contamination through higher sanitation requirements, greater use of terminal heaters or other acceptable pasteurization or bactericidal procedures.

We recommend that the uniform quality control guidelines, uniform sampling, and uniform testing methods recommended by the American Association of Veterinary Laboratory Diagnosticians be disseminated, accepted, and followed by all involved in the animal by-product feed industry.

The industry, however, is concerned with the problem of private laboratories not following the recommended guidelines of this Association for the isolation and detection of salmonella organisms in rendering plant by-products. The Committee will investigate and study this problem and make a specific recommendation next year in the hopes of correcting this situation.

The Committee is pleased to note that Consumer and Marketing Service has recognized the importance of the possibility of Salmonella contamination in meat and poultry products; and that inherent food poisoning problems could move from the consumer's kitchen to manufacturing establishments. We commend the Consumer and Marketing Service for the adoption of specific requirements directed particularly at cooked, ready-to-eat meat, and poultry products. We urge the various states to consider adoption of similar requirements.

Changes in the Salmonella Uniform Methods and Rules are proposed to:
1. Make the definitions used for animal products identical to those established by the Association of American Feed Control Officials.
2. Include definitions for some additional terms where there has been shown to be some ambiguity.
3. To provide for an alternate sampling schedule where the recommended sampling schedule cannot be followed.

The Salmonella program has been initiated with little additional funding by the individual states. Federal appropriations during FY 1969 were approximately $500,000. Twenty-six percent of which was used for serotyping and service support to private and public laboratories. The States are urged to use the Uniform Methods and Rules as a management tool in allotting available manpower and resources in efforts to control Salmonella in feed ingredients. This Committee urges the Animal
Health Division, USDA, to develop a statistical basis for reducing their inspection and sampling for those plants that have demonstrated an ability to consistently produce a product in which Salmonella is not detected by our program procedures.

The following should be expanded based on Committee discussion at the meeting:

a. Each member of the Salmonella Committee has been furnished with a copy of the National Academy of Sciences, “An Evaluation of the Salmonella Problem.” It is suggested the Committee make recommendations based on the report.

b. It is suggested the Committee recommend increased attention be given to the inspection of fish meal and meat meal imported into the United States. Also recommend negative certification for Salmonella be required from the country of origin.

c. The Committee should recognize the excellent cooperation between FDA, USDA, and individual states in avoiding duplication of efforts in the conduct of regulatory responsibilities in rendering and fish meal plant inspections and sample collections.

d. The Committee should consider aspects other than rendering plant programs for Salmonella Control. Giving recognition to the fryer-roaster turkey project in Minnesota, the cattle feedlot project in Kansas and the Dillon Beach project in California, what should be the future approach to Salmonella programs?

e. The Committee should maintain liaison with the Poultry Disease Committee concerning the eradication of Pullorum Disease and Fowl Typhoid.

FDA Animal By-Product for Feed Analytical Results (Domestic)
FY 1969
July 1, 1968 Through June 30, 1969

Total Number Samples Domestic Animal By-Products – 78
Reports:

- 10 tests positive 5
- 9 tests positive 4
- 8 tests positive 2
- 7 tests positive 3
- 6 tests positive 2
- 5 tests positive 5
- 4 tests positive 6
- 3 tests positive 8
- 2 tests positive 10
- 1 test positive 9
- 10 tests negative 24

Total 78

FDA Testing of Import Lots Fishmeal

Total Number Lots Examined 223
Total Number Lots Detained 12
FDA Testing of Import Lots Tankage, Bone Meal, Etc.

Total Number Lots Examined  31
Total Number Lots Detained  13

SALMONELLA UNIFORM METHODS AND RULES

Part I: Definitions

A. Animal Products — Blood meal, meat by-products, meat meal, meat and bone meal, meat meal tankage, whale meat, animal liver meal, animal liver and glandular meal, extracted animal liver meal, animal by-product — processed, poultry by-product meal, poultry hatchery by-product, dried meat solubles, poultry parts, poultry by-products, hydrolyzed poultry feathers, fleshings hydrolysate, whole eviscerated chicken, hair hydrolyzed, leather meal hydrolyzed, or blended mixtures thereof, to be used in animal feeds.

B. The definition for products listed in paragraph A of this part shall be those published in the current edition of the Association of Feed Control Officials Manual.

C. Lot — Paragraph change from H to C. No change in definition which reads:

Lot — A lot of animal product shall be the amount of product for a single shipment or a day's production, whichever is greater.

D. Official Sample — Paragraph change from I to D. The definition which currently reads:

I. Official Sample — A random sample of a finished product, ready for shipment, collected by a State or Federal inspector, in such a manner that it represents the contents of a total lot, which consists of ten (10) sample units with each sample unit of not less than 100 grams each, selected at random from each 1/10 section of the total lot. Each sample unit collected shall be individually packaged and identified.

Shall be amended to read:

D. Official Sample — A random sample of a finished product, ready for shipment, collected by a State or Federal inspector, in such a manner that it represents the contents of a total lot. (The recommended sample size is given in Paragraph F).

E. Plant Sample — A new paragraph to read:

Plant Sample — A random sample of a finished product, ready for shipment, collected by plant personnel in such a manner it represents the contents of a total lot.

F. Recommended Sample Size — A new paragraph. Place that section of Paragraph I in the definition of an official sample in a separate paragraph to read as follows:
Recommended Sample Size — The recommended sample shall consist of 10 sample units weighing not less than 100 grams each, selected at random from each 1/10 section of lot. Each sample unit collected shall be individually packaged and identified.

G. Acceptable Sample Size — a new paragraph to read:
Acceptable Sample Size or Alternate Sample — Any combination of 30 consecutive sample units, weighing not less than 100 grams each, collected by a State or Federal inspector on three or more separate inspections within a 12-month period in which not more than 10 sample units are collected on any one inspection. (This sample may be substituted for the recommended sample described in F above.)

H. Positive Sample — paragraph change from J to H. No change in definition which reads:
Positive Sample — A sample unit of an official sample from which Salmonella is recovered by an official laboratory.

I. Negative Sample — Paragraph change from K to I. No change in definition which reads:
Negative Sample — A sample unit of an official sample from which no Salmonella is recovered by an official laboratory.

J. Salmonella Tested — Paragraph change from L to J. No change in definition which reads:
Salmonella Tested — The laboratory examination of an official sample of product, collected as outlined in I above, and tested by the procedures and methods recommended by the USAHA and the U.S. Department of Agriculture for cooperative program activities. (ARS 91-68)

K. Rendering Establishment — Paragraph change from M to K. No change in definition which reads:
Rendering Establishment — An establishment that processes animal products as defined in A (this part) for use in animal feeds, whether or not operated in conjunction with a slaughtering or processing plant.

L. Blending Establishment — Paragraph change from N to L. No change in definition which reads:
Blending Establishment — An establishment that grinds, blends, mixes, or further processes animal, marine, or animal and marine protein for the purpose of making such proteins suitable for use in animal feeds.

M. Quality Control Program — Paragraph change from O to M. No change in definition which reads:
Quality Control Program — The sum of methods and procedures employed by an establishment designed to prevent, detect and eliminate Salmonella contamination in its finished product, as outlined in the Recommended Sanitation Guidelines,
and includes a test for Salmonella of a representative or composite representative sample of not less than one of the lots produced each week.

N. Approved Rendering or Blending Establishment — Paragraph change from P to N. The definition which currently reads:

Approved Rendering or Blending Establishment — A rendering or blending establishment that has been given official recognition for consistently producing a negative product and operating in compliance with the Recommended Sanitation Guidelines as described in Part II, paragraph C, below.

Shall be amended to read:

Approved Establishment — A rendering or blending establishment that has been given official recognition by the cooperating State and Federal agencies for having met the requirements for designation as an approved establishment as described in Par II, paragraph C.

O. Finished Product — (A new paragraph to read) Rendered material, ready for shipment, collected in the shipping or storage area, and will undergo no further processing in the plant where collected.

P. Negative — (A new paragraph to read) Salmonella not detected in the finished product when sampled at the rate and examined according to the procedures specified for the program.

Part II — Recommended Procedures

Paragraph A Evaluation Phase which currently reads:

A. Evaluation Phase

1. Objectives:
   a. Determine the presence or absence of Salmonella in the finished product produced by each plant in the state.

Shall be amended to read:

A. Phase I, Evaluation:

1. Objectives:
   a. Determine the presence or absence of Salmonella in detectable amounts in the finished product produced by each plant in the state.

Subparagraph 1 b, c, and 2 a, which reads as follows has no change:

b. Determine the plants that are operating in compliance with the Recommended Sanitation Guidelines.

c. Determine the plants that are not operating in compliance with the Recommended Sanitation Guidelines.

2. Classification of Plants:
   a. Plants shall be classified as positive or negative based upon the results of laboratory examination of official samples.

Subparagraph 2 b, which reads:

b. Positive classification — A positive classification shall result from one or more positive sub-samples in an official sample on a single test.
shall be amended to read:

b. Positive classification — A positive classification shall result from one or more positive sample units (sub-samples) in an official sample on a single test.

Subparagraph 2 c, which reads:

c. Negative classification — A negative classification shall result from negative tests for all sub-samples on three consecutive tests of official samples. The tests shall be conducted at an interval of not less than 30 days apart.

shall be amended to read:

c. Negative Classification — A negative classification shall result from a negative test of the sample units in an official sample of the recommended sample size or the alternate acceptable sample size. The 30 sample units shall be collected on three or more separate inspections within a three to twelve month period. Not more than 10 sample units shall be collected on any one inspection.

Subparagraph 3 — Procedures which reads:

3. Procedure:

Conduct three inspections of each plant not less than 30 days apart and the bacteriological examination of an official sample of finished product collected during each inspection.

shall be amended to read as follows:

3. Procedure:

Conduct not less than three inspections of each plant at an interval of not less than 30 days apart and submit to an official laboratory for bacteriological examination the sample units in an official sample of finished produce of the recommended sample size or alternate sample size. The sample units shall be collected during a period of not less than 90 days nor more than one year.

Paragraph B — Cleanup Phase which currently reads — B. Cleanup Phase, is amended to read as follows:

B. Phase II, Cleanup

Subparagraphs 1 a, b, 2 a, (1), (2), (3), and (4) which currently reads as follows have no change:

1. Objectives:

   a. Reduce the number of positive plants.
   b. Increase the number of negative plants that remain negative.

2. Procedures:

   a. Positive Plants

      (1) Conduct an extensive epidemiological study of each positive plant in an effort to identify the source or sources of contamination.
      (2) Make recommendations to management to eliminate areas of contamination or potential contamination.
      (3) Make recommendations to management from bringing operations into compliance with the Sanitation Guidelines.
      (4) Assist plant in developing a quality control program as related to
Salmonella contamination.

Subparagraph 2 b, (1) which currently reads:

b. Negative plants
   (1) Conduct three inspections at not less than thirty day intervals
       until the plant has received three consecutive negative tests.
       Subsequent inspections and tests shall be conducted at not less
       than 120 day intervals.

Shall be amended to read as follows:

(b) Negative plants
   (1) Inspections and tests shall be conducted at intervals of not more
       than 120 days. Samples shall be collected in such a manner that
       30 sample units are collected each 12 month period and not more
       than 10 finished product samples collected on any one inspec-
       tion.

Subparagraph 2 b, (2), (3), and (4) which read as follows have no change:

(2) Make recommendations to management to eliminate areas of
    contamination or potential contamination.
(3) Make recommendations to management to bring operations into
    compliance with the Sanitation Guidelines.
(4) Assist plant in developing a quality control program as related to
    Salmonella contamination.

Paragraph C 1, a, which reads as follows:

C. **Approved Phase**
   1. Objectives:
      a. To give recognition to plants that demonstrate the capability of
         consistently producing a product negative for Salmonella when
         sampled and examined by the procedures recommended by the
         USAHA and the U.S. Department of Agriculture for cooperative
         program activities.

Is amended to read:

C. **Approved Phase**
   1. Objectives:
      a. To give recognition to plants that operate in compliance with the
         Sanitation Guidelines and demonstrate the capability of consistently
         producing a product negative for Salmonella when sampled and
         examined by the procedures recommended by the USAHA and the
         U.S. Department of Agriculture for cooperative program activities.

Subparagraph C 1 b, 2 which reads as follows has no change:

b. To encourage management to attempt to maintain the status of clean
   plants.

2. Procedures for qualification:
   A plant may qualify for designation as an approved establishment
   provided:

Subparagraph 2 a which reads as follows:

a. It has had three (3) consecutive negative tests of official samples
   conducted at intervals of not less than 30 days apart.
REPORT OF

Shall be amended to read:

a. It has had negative tests of 30 consecutive sample units collected in not less than 90 days and not more than 10 sample units were collected on any one inspection.

Subparagraph 2 b, c, d which reads as follows had no change:

b. It is operating in compliance with the Recommended Sanitation Guidelines (ARS 91-47), as verified by a written inspection report by a State or Federal inspector.

c. It has established a quality control program which meets the approval of the cooperating State and Federal animal health officials.

d. The management applies for approved status and approval is granted by the cooperating State and Federal animal health officials.

Subparagraph C 3 a, which reads as follows:

3. Procedure for maintenance:

a. A plant may maintain its approved status provided a negative test of an official sample is conducted at an interval of not more than 120 days.

Shall be amended to read:

3. Procedure for maintenance:

a. A plant may maintain its approved status provided a negative test is obtained of any contamination of 30 sample units collected during a 12 month period and not more than 10 sample units are collected on any one inspection.

Subparagraph 3 b (1), which reads as follows has no change:

b. In the event an approved plant has a positive test of an official sample, it shall:

(1) Follow the procedures outlined for positive plants in the cleanup phase (paragraph B-2, this part).

Subparagraph 3 b (2), which reads as follows:

(2) Obtain three (3) consecutive negative tests of official samples within a six-month period. The interval between tests shall be not less than 7 days apart.

Shall be amended to read:

(2) Within six months, obtain a negative test of an official sample of the recommended or alternate sample size. The sample units shall be collected during a period of not less than 21 days, and not more than 10 sample units collected on any one inspection.

Subparagraph 3 (c) and (b) which read as follows have no change:

c. It is operating in compliance with the Recommended Sanitation Guidelines (ARS 91-47) as verified by a written inspection report by a State or Federal inspector.

d. It has established a quality control program which meets the approval of the cooperating State and Federal animal health officials.
THE ROLE OF THE REGULATORY VETERINARIAN
IN ANIMAL RESEARCH

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Let us start taking apart the topic and then reconstruct it. First change the word "role" to function; next drop the words "animal research" and we have the question "What is the function of the regulatory veterinarian?" Dropping "regulatory", we are left with the subject, "What is the function of the veterinarian?"

A brief historical review of the development of veterinary medicine may help us answer our new question. Cockrill\(^1\) states in his paper *International Trends in Veterinary Medicine*, "The attitude of the profession itself is undergoing drastic alteration as our status improves and we are appreciating the advantages to be gained . . . This is a complex field and emphasis must be placed on the control of diseases which, being intercommunicable between animals and man, pose a question of public health as well as one of economic importance." Cockrill's belief of 1964 that the veterinary profession is in a status that needs improvement is worthy of additional study, for in the same paragraph he alludes to the public health aspects of veterinary medicine. Let us turn our attention to remarks made by J. F. Smithcor's\(^3\) article entitled *Development of Veterinary Medical Science*. "Along with the other arts and sciences, veterinary medicine was esteemed by ancient people. About 2200 B.C. specialization of practice reached a peak in ancient Egypt where mention is made of "doctors of fowls," as well as doctors of other species. Veterinary medicine was a learned profession in Egypt, practiced side by side with that of medicine. The Romans, however, despised medicine and had no physicians of their own for several centuries, depending on itinerant Greeks. With the decline of civilization following the fall of the Roman Empire, veterinary medicine — along with all the other arts and sciences — descended into the morass of the Dark Ages. As early as the fifth century Vegetius Renatus in his "Artis Veterinariae," deplored the lowly status of this once proud profession, claiming it should be second only to that of human medicine. The latter began to regain its status in the early Middle Ages, the veterinary art continued to flounder. In British writings, for example, the term "veterinary" was virtually unknown until the late Eighteenth Century."

Such historical reviews of the veterinary profession allow us to see the changing status of the profession from one period of history to another. It has gone from a status of one of the leading professions to one of the lower groups being looked upon with distaste. However, the veterinary profession is that art of medicine responsible for improving the health status of the animal kingdom. This is all-encompassing and it's the public health aspects that crosses over and reaches into the area of human medicine. F. H. Top\(^4\) ably summarizes this relationship by stating, "Human medicine and veterinary medicine cannot be divorced . . . Members of the veterinary and human medical professions need to consider the tremendous importance of one to the other . . . Herein lies the veterinarian's responsibility . . . and opportunity. Is there a need to return to the philosophy of the Greeks and have specialization, such as "doctors of fowls"? Is the burden of mastering the knowledge that has been accumulated relating to one species too great for any one
individual to master, much less consider the total animal kingdom?

As the veterinary profession would sink from its higher status to its lower status, there has always been a group to carry on and practice the art with scientific knowhow and knowledge that is needed to give it the status of a profession. In due time the contribution to man were surely recognized and the profession would start returning to its rightful status. This, I think, cannot be taken too lightly and we must recognize that for our profession to maintain its status, not merely for the purpose of having pridelful, selfish dignity, but to be able to render its greatest service, it must function in a learned manner. It will require the knowledge related to the sciences, as well as the ability to apply this knowledge in an artful manner. It truly must be looked upon as an art and a science.

The answer to our first question, "What is the role or function of the veterinarian?" may be — the application of veterinary medical knowledge for the benefit of mankind.

Returning the word "regulatory" gives us the question, "What is the role of the regulatory veterinarian?"

The regulatory veterinarian has served the citizens of the United States for a number of decades. Some of the familiar areas include: international movement of animals, importation of animal by-products, importation and interstate movement of organisms and vectors, veterinary biologics and disease control programs, such as tick fever and tuberculosis. Government programs are necessary for the protection of the nation's animal health, and, in turn, the health of the nation's people.

United States Regulatory Veterinary Medicine has established many areas of improvements to elevate the status of the veterinary profession. Movement of animals and animal by-products prevents the introduction of such diseases as hoof and mouth. Disease control programs have allowed several animal diseases to be controlled and/or eradicated. Smith and Kilborne's outstanding work with bovine piroplasmosis served as a working model to allow others to control diseases such as yellow fever. The Bureau of Animal Industry has used a team composed of a variety of disciplines as it solved a number of very important medical problems.

"The Function of the Regulatory Veterinarian" may be defined as the application of veterinary medicine to protect or improve animal health as required by law.

We are now ready to consider our printed title — "The Role of the Regulatory Veterinarian in Animal Research." A federal law relating to animals in research was passed August 24, 1966, the Animal Welfare Act. Passage of this law followed many years of hearings relating to the need for such legislation. To understand the reason for and the value of such a law requires a short historical review of animal usage in research.

Three aspects of such usage will be considered — the philosophy of using animals, scientific use of animals, and the legal use of animals.

The societies of the world differ widely regarding their beliefs concerning how animals should relate to man in this world. Between the extreme beliefs of the vegetarian and the cannibal we find the majority of mankind. It is told that some cannibal tribes became so ruthless in the destruction of the human species that some of their group established the practice of vegetarianism to save the tribal children. Destruction of animal life, be it insects or mammals, is not done by those
who believe reincarnation occurs. The belief of our Judeo-Christian society places man as the supreme animal having dominion over all other animals. Such a philosophical position has allowed man to use animals in such areas as power, transportation, nutrition, pets and research. Minority groups still take exception to using animals, and if the logic used by the majority of our society cannot change the beliefs of these people, I suggest we meet after death to find out who was right. Unanimity of opinion will be difficult to attain in these philosophical areas and we will need to accept the majority rule.

Even though our society kills animals for food and clothing, it must be viewed as a humane society. People of the United States have developed better and more humane use of animals as the wilderness was rolled back, making man’s and animal’s life easier.

The objectives of scientific groups reveal the planning for development of our knowledge of animal life. As our understanding of animal life grows, the pain and suffering of animals decreases.

Antivivisectionism has not attracted a large number of followers. People of position, such as Queen Victoria of England, were reported by Dennis\(^2\) to be a champion of antivivisectionism.

Antivivisectionists have done an excellent job of promoting division between the scientific organization and humane groups. In reality, both are searching for a means to aid animals. The antivivisectionist has told half truths, has taken statements out of context and insinuated unfavorable acts or conditions to promote his cause.

The philosophic and scientific ideas relating to man’s use of animals is a complex subject. It is not surprising that some of the public can be misled by such trickery.

In 1863, Henry Bergh established the American Society for the Prevention of Cruelty to Animals in New York. New York passed the first anticruelty law of the United States and Bergh’s society enforced these. This excellent humane group makes on-site visits to most of New York State’s research laboratories.

A number of other groups have been organized varying in their degree of concern for animals used in research. As these groups developed methods of belittling animal research, some of the scientific people responded, but the majority of scientists felt that they had more important work to do and would not take time to even discuss their positions.

The National Society of Medical Research was organized and made an effort to inform law makers about both view points. More recently, the NSMR has made an additional effort to help inform the public about the benefits man receives from animal research. This effort to educate the public is to be commended.

At no time has the scientific community been satisfied with its knowledge of animal care. As today’s research is completed it reveals changes needed for tomorrow’s improvements. A number of United States scientific societies have been working for decades with animals, such as the American Veterinary Medical Association, and agricultural bodies located at land-grant colleges and experimental stations. More recently, a group of Chicago scientists formed the Animal Care Panel. This name has been changed to the American Association for Laboratory Science but the objectives are still the same. AALAS is a nonprofit educational
association of persons and institutions professionally engaged in the production, care and study of laboratory animals.

The success of the Animal Care Panel in increasing and disseminating knowledge of animals can be seen in most animal laboratories in the United States. The quality of health and the method of using the animals as biological models today compared to conditions twenty years ago is a witness to the success of AALAS.

The veterinary profession has recognized the need for specialists in Laboratory Animal Medicine. A number of veterinarians explained the need for a College of Laboratory Animal Medicine to the AVMA. Approval followed and the College was formed in 1957.

The activities of the Institute of Laboratory Animal Resources, an agency of the National Academy of Science — National Research Council began in 1952. The major categories ILAR have developed are: develop and disseminate information, establish scientific standards for animal care and management, and prepare educational materials and curricula for laboratory animal personnel. ILAR has done an excellent job in all of these areas.

As these various groups (many equally good groups have been omitted from our discussion) succeeded in their efforts to improve animal research, the discussion concerning needed legislation to improve animal care and/or stop the use of animals in research continued.

The Animal Welfare Act of PL 89-544 was a result of the controversy. The Animal Welfare Act established a legal reference relating to the use of animals in research. This federal law authorized the Secretary of Agriculture to regulate the transportation, sale and handling of dogs, cats and certain other animals intended to be used for purposes of research or experimentation and for other purposes. The law first asked the secretary to establish and promulgate standards which include adequate veterinary care.

The veterinarians in the USDA were given the responsibility of regulating PL 89-544. The question, "What is the role of the regulatory veterinarian in animal research?" was asked by many at this time. To have had so many groups, each firmly convinced of their position, arguing about the definition of animal welfare and the role of animals in research put this group of veterinarians in a very tenuous position.

The USDA is to be commended for executing the law in a manner which has been satisfactory to most of the critics.

First, a search was made for information to assist in establishing the standards. Professional judgment based upon a sound knowledge of veterinary medicine was then used to select the standards that are now employed.

Once the tangible factors have been established, such as feeding, watering, etc., judgment of the veterinary profession is needed to keep the standards parallel with scientific advances.

The Animal Welfare Act explicitly requires adequate veterinary care be provided these animals. This area of professional responsibility is more difficult to define. The responsibility might be easily avoided by determining if the inspected organization has in its employment a graduate veterinarian.

Regulatory veterinarians cannot assume the law's requirement of adequate
veterinary care is being provided merely by the presence of a professional on a payroll. The law asks for judgment to be made. The evaluation of veterinary care must be conducted in a spirit of professional cooperation between the regulatory veterinarian and the veterinarian at the premises being inspected. A distinction must be made concerning quantity and quality of professional service. Some groups may need to employ more professional time to assure adequate care needs are being met. A shortage of veterinarians exists and the solution of this problem may be very difficult. If the Veterinarian's quality of professional judgment is questionable, the regulatory veterinarian will need to correct these misunderstandings.

Professional differences of opinion may first stem from the definition of "adequate veterinary care". Use of animals...acute vs. chronic studies, evaluation based upon one factor vs. many factors...will determine the degree of perfection needed. Therefore, an individual fortunate enough to have only needed and used extremely healthy animals may not recognize the validity of lesser standards. The minimum standard must relate to the comfort of the animal. Under no condition should breeder, dealer or conditioning programs allow animals to suffer. For example, all will agree that a prevention medicine program is needed to control intestinal parasites in a canine breeding program. The degree of freedom then becomes the debatable question. The pathologist would like complete absence of scarring produced by migrating larvae. The surgeon, having different interests, would not be as concerned with tissue changes if the animal had recovered clinically.

After semantical differences have been resolved, the regulatory veterinarians may need to settle differences in the evaluation of scientific matters. Any number of practical important problems can be considered at this time. How should canine viral diseases be controlled in a dealer's facilities? Many of these dogs have been exposed to virulent virus before purchase by the dealer. Should a vaccination program be established? If so, should the detailed plan be recommended by the regulatory veterinarian or the veterinarian at the dealer's site? Few veterinarians agree upon all aspects of canine distemper; therefore, immediate differences in professional judgment should be expected. Zoonotic diseases are especially disturbing; that is, their effects cause animal suffering, reduce the usefulness of the research animals, and create public health hazards. The regulatory veterinarian must have a scientific method established for settling professional opinion differences.

Historically we know the successful leaders of our profession artfully applied scientific knowledge. Problems mean that a hypothesis must be stated, a plan to prove it developed, and an experiment must be conducted and the results of the experiment must be evaluated.

Money is needed to resolve many of these problems. Until funds to support this research is available, the regulatory veterinarian will have to accept veterinary procedures commonly accepted by the profession, even though his personal views do not concur. When accepted procedures are not used, the regulatory veterinarian must challenge his colleagues. The basis of professional challenging must relate to the diagnosis of medical problems. The regulatory veterinarian should be supplied the data collected by the challenged colleague. If additional information to make proper diagnosis is required, secure it. Make use of veterinary knowledge. This may mean a lot of extra homework for the regulatory veterinarian who has not worked
with all the common species of laboratory animals.

SUMMARY

Veterinary medicine has been held in high esteem only when its leaders' application of the art was based upon maximum use of scientific knowledge. Regulatory veterinarians are conducting themselves in a similar manner artfully applying scientific knowledge. Professional people are needed to establish and keep legal standards in accordance with scientific knowledge. In addition, determination of adequate veterinary care must be a result of professional judgment based upon sound diagnostic methods. Under no circumstances should the regulatory veterinarian assume his role is that of a layman who can, with less training, apply or measure standards established by the more highly trained professional.

REFERENCES


As mentioned in last year’s report, the charge to this Committee was to develop a model animal welfare bill that could be used by states that are contemplating introducing this type legislation. It was recognized by the Committee that PL 89-544 provided basic authority in this area and that this bill would extend coverage to include pet shops, pounds and shelters.

The Committee met on four occasions and the final draft of a model act will be submitted to the Executive Committee today or tomorrow for its approval. It is also our understanding that if approved, the Executive Committee would consider forwarding the act to the Council of State Governments as recommended state legislation.

The purpose would be to provide a basis for states that do not have such legislation and to further uniformity in state legislation that apply to animal welfare.

Committee members were also made aware of legislation that has been introduced in Congress pertaining to the “soring” of horses. The Committee drafted a resolution to the Executive Committee supporting the intent of such legislation when it applies to all breeds of horses.

“DRAFT OF A PROPOSED MODEL STATE LAW”

October 15, 1969

Title: A proposed Model Law designed to supplement the Federal Laboratory Animal Welfare Act (P.O. 89-544), to provide standards for the care of animals in animal shelters, pounds and pet shops, and to regulate dealers and dog wardens.

Section 1. Purpose: (1) To protect the owners of dogs and cats from the theft of such pets; (2) to prevent the sale or use of stolen pets; (3) to insure that all warm-blooded vertebrate animals, as items of commerce are provided humane care and treatment by regulating the transportation, sale, purchase, housing, care, handling and treatment of such animals by persons or organizations engaged in transporting, buying, or selling them for such use; (4) to insure that animals confined in pet shops, kennels, animal shelters, auction markets and pounds are provided humane care and treatment; (5) to release for sale, trade or adoption only those animals which appear to be free of infection, communicable disease, or abnormalities, unless veterinary care subsequent to release is assured.

Section 2. Definitions: As used in this article and the regulations promulgated
thereunder, the following terms shall be construed, respectively, to mean:

(a) "Pound" or "dog pound" means a facility operated by a state, or any political subdivision thereof, for the purpose of impounding or harboring seized, stray, homeless, abandoned or unwanted dogs, cats, and other animals; or a facility operated for such a purpose under a contract with any municipal corporation or incorporated society for the prevention of cruelty to animals.

(b) "Person" means any individual, partnership, firm, joint stock company, corporation, association, trust, estate, or other legal entity.

(c) "Animal Shelter" means a facility which is used to house or contain animals and which is owned, operated, or maintained by a duly incorporated humane society, animal welfare society, society for the prevention of cruelty to animals, or other non-profit organization devoted to the welfare, protection and humane treatment of animals.

(d) "Pet Shop" means an establishment where animals are bought, sold, exchanged, or offered for sale or exchange to the general public at retail. This shall not include an establishment or person whose total sales are the offspring of not more than five canine or feline females maintained on their premises.

(e) "Kennel" means a place or establishment other than a pound or animal shelter where animals not owned by the proprietor are sheltered, fed and watered in return for a fee.

(f) "Animal" means any dog or cat, rabbit, rodent, nonhuman primate, bird or other warm-blooded vertebrate but shall not include horses, cattle, sheep, goats, swine and domestic fowl.

(g) "Public Auction" means any place or location where dogs or cats are sold at auction to the highest bidder regardless of whether such dogs or cats are offered as individuals, as a group, or by weight.

(h) "Commissioner" means the Commissioner of Agriculture of the State of

(i) "Dealer" means any person who sells, exchanges, or donates, or offers to sell, exchange, or donate animals to another dealer, pet shop, or research facility, providing, however, that an individual who breeds and raises on his own premises no more than a single litter of pups or kittens per year, unless bred and raised specifically for research purposes shall not be considered to be a dealer for the purpose of this article.

(j) "Research facility" means any place, laboratory or institution at which scientific tests, experiments, or investigations involving the use of living animals are carried out, conducted, or attempted.

(k) "Primary enclosure" means any structure used to immediately restrict an animal or animals to a limited amount of space, such as a room, pen, cage, compartment or hutch.

(l) "Housing facility" means any room, building, or area used to contain a primary enclosure or enclosures.

(m) "Sanitize" means to make physically clean and to remove and destroy to a practical minimum, agents injurious to health.

(n) "Euthanasia" means the humane destruction of an animal accomplished by a method that involves instantaneous unconsciousness and immediate death or by a method that involves anesthesia, produced by an agent which causes painless loss of
consciousness, and death during such loss of consciousness.

(o) "Ambient temperature" means the temperature surrounding the animal.

(p) "Adequate feed" means the provision at suitable intervals — not to exceed 24 hours — of a quantity of wholesome foodstuff suitable for the species and age, sufficient to maintain a reasonable level of nutrition in each animal. Such foodstuff shall be served in a sanitized receptacle, dish, or container.

(q) "Adequate water" means a constant access to a supply of clean, fresh, potable water provided in a sanitary manner or provided at suitable intervals for the species and not to exceed 24 hours at any interval.

(r) "Dog warden" means any person employed, contracted or appointed by the state or any political subdivision thereof for the purpose of aiding in the enforcement of this law or any other law or ordinance relating to the licensing of dogs, control of dogs, or seizure and impoundment of dogs and includes any state or municipal peace officer, animal control officer, sheriff, constable or other employee whose duties in whole or in part include assignments which involve the seizure or taking into custody of any dog.

Section 3. No municipality shall operate a dog pound for more than six months subsequent to the effective date of this article unless a certificate of registration for such dog pound has been granted by the commissioner. Application for such certificate shall be made in the manner provided by the commissioner. No fee shall be required for such application or certificate. Certificates of Registration shall be valid for a period of years or until revoked and may be renewed for like periods upon application in the manner provided.

Section 4. No person shall operate an animal shelter for more than six months subsequent to the effective date of this article unless a certificate of registration for such animal shelter has been granted by the commissioner. Application for such certificate shall be made in the manner provided by the commissioner. No fee shall be required for such application or certificate. Certificates of Registration shall be valid for a period of years or until revoked and may be renewed for like periods upon application in the manner provided.

Section 5. No person shall operate a pet shop as defined in this article for more than six months subsequent to the effective date of this article unless a license to operate such establishment shall have been granted by the commissioner. Application for such license shall be made in the manner provided by the commissioner. The license period shall be the calendar (or fiscal) year and the license fee shall be $25 (or $50 or $100), for each license period or part thereof beginning with the first day of the calendar (or fiscal) year.

Section 6. No person shall operate a public auction or a kennel as defined in this article for more than six months subsequent to the effective date of this article unless a license to operate such establishment shall have been granted by the commissioner. Application for such license shall be made in the manner provided by the commissioner. The license period shall be the calendar (or fiscal) year and the license fee shall be $25 (or $50 or $100) for each license period or part thereof.
beginning with the first day of the calendar (or fiscal) year.

Section 7. No person shall be a dealer as defined in this article after the first day of the calendar (or fiscal) year unless a license to deal shall have been granted by the commissioner to such person. Application for such license shall be made in the manner provided by the commissioner. The license period shall be the calendar (or fiscal) year and the license fee shall be $100 (or $200 or $500) for each license period or part thereof beginning with the first day of the calendar (or fiscal) year.

Section 8. A certificate of registration may be denied to any pound or animal shelter and a license may be denied to any public auction, kennel, pet shop or dealer or, if granted, such certificate or license may be revoked by the commissioner if, after public hearing, it is determined that the housing facilities and/or primary enclosures are inadequate for the purposes of this article or if the feeding, watering, sanitizing and housing practices at the pound, animal shelter, public auction, pet shop or kennel are not consistent with the intent of this article or with the intent of the rules and regulations which may be promulgated pursuant to the authority of this article.

Section 9. Operation of a pet shop, kennel or public auction without a currently valid license shall constitute a misdemeanor subject to a penalty not less than $_______ and not more than $_______, and each day of such operation shall constitute a separate offense.

Section 10. Dealing in animals as defined in this article without a currently valid license shall constitute a felony subject to a penalty not less than $_______ and not more than $_______, or confinement for a period not to exceed _______ months or both. Continued illegal operation after conviction shall constitute a separate offense. Animals found in the possession or custody of an unlicensed dealer shall be subject to immediate seizure and impoundment and, upon conviction of such dealer, shall become subject to sale or euthanasia at the discretion of the commissioner.

Section 11. Failure of any person licensed or registered to adequately house, feed and water animals in his possession or custody shall constitute a misdemeanor, and upon conviction the person shall be subject to a fine of not less than ($2, or $5, or $10) per animal or more than a total of ($1,000, or $2,000). Such animals shall be subject to seizure and impoundment and upon conviction of such person may be sold or euthanized at the discretion of the commissioner and such failure shall also constitute grounds for revocation of license or registration after public hearing.

Section 12. The governing body of the political subdivision regulating the operation of a pound shall determine the method of disposition of animals released by such pound. Any proceeds deriving from such gift, sale, or delivery shall be paid directly to the clerk or treasurer of the political subdivision and no part of such proceeds shall accrue to any individual. The board of directors of an incorporated humane society shall determine the method of disposition of animals released by its animal
shelter. Any proceeds deriving from such gift, sale, or delivery shall be paid directly to the clerk or treasurer of the humane society and no part of such proceeds shall accrue to any individual.

No dog warden shall give or sell or negotiate for the gift or sale to a pet shop, dealer, or research facility of any animal which may come into his custody in the course of carrying out his official assignments. No dog warden shall be granted a dealer's license and each application for such license shall include a statement made under oath, that neither the applicant or any member of employee of the firm, partnership, or corporation making application is a dog warden within the meaning of the definition herein. A dog warden, upon taking custody of any animal in the course of his official duties, shall immediately make a record of the matter in the manner prescribed by the commissioner and the record shall include a description of the animal including color, breed, sex, approximate weight, reason for seizure, location of seizure, the owner's name and address if known and all license or other identification numbers if any. Complete information relating to the disposition of the dog shall be added in the manner provided by the commissioner immediately after disposition.

Section 13. Violation of any provision of this article which relates to the seizing, impoundment and custody of an animal by a dog warden shall constitute a misdemeanor subject to a fine not less than $________ and not more than $________, and each animal handled in violation shall constitute a separate offense.

Section 14. The commissioner may, as he deems indicated and appropriate, promulgate rules and regulations consistent with the objectives and intent of this article for the purpose of carrying out such objectives and intent. Such rules and regulations may include, but are not limited to provisions relating to humane transportation to and from registered or licensed premises, records of purchase and sale, identification of animals handled, primary enclosures, housing facilities, sanitation, euthanasia, ambient temperatures, feeding, watering, and adequate veterinary medical care. He may at his discretion, after public hearing, adopt in whole or in part, those portions of the rules and regulations promulgated by the Secretary of the United States Department of Agriculture pursuant to the provisions of United States Public Law 89-544, commonly known as the Laboratory Animal Welfare Act, which are consistent with the intent and purpose of this article.

Section 15. This article shall not apply to a place or establishment which operated under the immediate supervision of a duly licensed veterinarian as a hospital where animals are harbored, boarded and cared for incidental to the treatment, prevention, or alleviation of disease processes during the routine practice of the profession of veterinary medicine except that, if animals are accepted by such place, establishment or hospital for the primary purpose of boarding in return for a fee, the place, establishment or hospital shall be subject to the kennel license provisions of this article and the regulation relating thereto which may hereafter be promulgated by the commissioner. This article shall not apply to any dealer or research facility during the period such dealer or research facility is in the
possession of a valid license or registration granted by the Secretary of Agriculture pursuant to the provisions of United States Public Law 89-544.

Section 16. For the purposes of administering and enforcing this article and the rules and regulations that may hereafter be promulgated thereunder the sum of $_______ is hereby appropriated.

Section 17. If any provision of this article or the application of any such provision to any person or circumstances shall be held invalid, the remainder of the article and the application of any such provision to persons or circumstances other than those as to which it is held invalid shall not be affected thereby.

Approved by the Executive Committee of
The United States Animal Health Association
in session at Milwaukee, Wisconsin
October 17, 1969
RESPONSIBILITIES OF THE PRACTICING VETERINARIAN WITH RESPECT TO THE WHOLESAEMONESS OF SLAUGHTERED LIVESTOCK AND POULTRY


The health of livestock and poultry certainly is of prime importance to all farmers, diarymen, ranchers, and feeders. The condition of health has a direct effect on the monetary return from any herd or flock whether the return is measured by the number of offspring produced, the rate of gain, the feed efficiency, or the milk or eggs produced. A progressive producer keeps a close surveillance over the health status of the animals in his herd or the birds in his flock.

Agricultural economists predicted the number of animals and birds on farms engaged in the production of livestock and poultry would increase to meet the consumer demand for meat and meat food products. Modern husbandry practices required to raise larger numbers of animals and birds in greater concentrations have many economic advantages, but increased the problems of sanitation and disease control. Confinement in a limited space for a period of time is conducive to disease outbreaks which can markedly increase production costs. Disease prevention is a vital approach in the management of these large agricultural enterprises in order to avoid catastrophes.

Livestock and poultry producers rely heavily on agricultural chemicals and drugs in overcoming the adverse conditions which alter efficient production and affect their economic returns. No successful producer questions the value of these chemicals and drugs. Many remember when the rate of gain was slower and their capital was tied up in an animal longer than it is today. They remember market weights less than what the same amount of feed requires today. Unquestionably, these chemicals and drugs have played an important role in the industry's success.

At the same time, in our society the consumer expects the meat and meat food products derived from these animals and birds to be acceptable for consumption, be nutritious, and to have met the standards for wholesomeness and adulteration.

Many animals and birds are slaughtered for human food when their productive usefulness has declined and disease can be a factor in this declining productivity. The veterinary practitioner is frequently asked for his opinion concerning the most economical method to handle those affected with a diseased condition. The veterinarian's diagnosis, management, and/or treatment of a herd or flock at this time may save or ruin the owner financially.

After the veterinarian has made an examination, the decision can either be that a course of treatment be given or they should be salvaged through slaughter. Salvage through slaughter is more difficult today because what appears suitable for human consumption may not be, if unsafe levels of a drug or chemical are present in the tissues. This additional factor of assuring wholesomeness must be evident in the veterinarian's decision.

One veterinarian may be responsible for advising the owners of thousands of head of poultry or livestock and could be directly responsible for the presence of chemical or drug residues in the edible products derived from these animals or birds. This could have a major effect on the food supply of a large number of
human beings. Under these circumstances, the veterinarian's decisions reach beyond economic considerations.

Some of the factors for the veterinarian to consider when making a decision regarding the most economical method of handling animals or birds affected with a disease condition are:

1. The practicing veterinarian should possess a working knowledge of the restrictions imposed on diseased and unsound animals and poultry during the inspection for wholesomeness at the time of slaughter for human food.

At antemortem inspection, livestock are placed into three categories depending on whether or not an abnormal condition exists. The animals may be condemned as unfit for human food; they may be showing clinical signs of an abnormal condition and be identified as a "suspect" for a more detailed examination; or they are passed for regular slaughter.

Let us review the federal meet inspection procedures to handle animals showing evidence of an abnormal condition at anti-mortem inspection.

A. Dying animals are marked "U.S. Condemned," humanely dispatched, and handled either by being tanked, denatured on the premises, or incinerated under the supervision of an inspector.

B. Animals which have been disabled, usually the result of an injury received by falling during loading and unloading, or from overcrowding in trucks, pens, etc., are, if they are unfit for human food, marked "U.S. Condemned," humanely dispatched, and handled as a dead animal by being tanked, denatured, or incinerated. Those animals which are not as severely injured are marked "U.S. Suspect" to be given a special examination by a veterinarian during the inspection for wholesomeness to determine their suitability for human consumption. However, these animals may have been on feeds containing drugs such as the feed additive diethylstilbestrol and must meet the required withdrawal periods to be suitable for human food.

C. Diseased animals as determined by a veterinarian can be marked as "U.S. Suspect" to be given a special examination during the post-mortem inspection. Those that are deemed unfit for human food because of a diseased condition such as listeriosis, pneumonia, grass tetany are marked "U.S. Condemned," humanely killed, and handled as a dead animal. It is also possible to set these animals aside for treatment. The treatment can be administered by the plant's veterinarian or from a private practitioner for the plant management. If these animals return to normal, they can be resubmitted for ante-mortem inspection. With permission from either a federal or state animal health official, an animal exhibiting a disease condition which has made it unfit for human food can be released back to the owner for treatment. Again, if the animal returns to normal health, it can be resubmitted for ante-mortem inspection. Lastly, diseased animals may be held for an examination by an animal health official for diagnostic reasons. This is in such cases where vesicular diseases and hog cholera may be suspected, or even a foreign animal disease.

D. At ante-mortem inspection, an animal is marked "U.S. Condemned" when showing signs of toxicity resulting from a chemical, a pesticide, or a drug. This animal could be humanely killed and tanked, denatured, incinerated or, depending on the causative agent, it could be held for observation and treatment. It is also possible to release this animal back to the owner with permission from an animal
health official. After the unacceptable residue has been eliminated from the animal's tissues, it can be resubmitted for ante-mortem inspection. Clinically, normal animals which have not completed a required withdrawal period and are unfit for human food because of a residue are held for the proper time period and resubmitted for ante-mortem inspection.

2. A second consideration is a treatment should not be given only to mask the signs of the disease condition to enhance its passing ante-mortem and post-mortem inspection.

Food hygiene veterinarians cannot condone injections that would result in an altered metabolism nor treatments of animals in a terminal condition resulting only in the masking of the severity of the condition to influence the decision regarding suitability for human consumption.

3. A third consideration is to recommend for slaughter only those which have responded to treatment and have passed the systemic phases of the disease.

4. A fourth consideration is if the animals or birds suffer from a condition that has made them obviously unfit for human food or they have a condition that has public health significance, a recommendation for slaughter must not be given.

5. A fifth factor for consideration is to use only approved chemicals and drugs and to observe all required withdrawal periods after treatment.

There are definitely correct and incorrect ways to use these substances. Consumers, as well as those of us who have a responsibility for consumer protection, are very much concerned with the misuse of these chemicals and drugs and their effects on the wholesomeness of food. A practicing veterinarian may not be familiar with the research that contributed to the approval for a chemical or drug to be used in livestock or poultry. He probably does not know the extent of the hazards from tissue residues or the margin of safety the drug has in sick animals or birds, or how effective the drug is in treatment of the disease. The label provides him with this information and these instructions must be followed without deviation or a misuse will result. One method to misuse a chemical or drug is overdosing — if one dose does good — two doses are twice as good. Improper mixture proportions on all drugs, including supplements, is another way to misuse. Unless the mixing is thorough, some animal or bird is bound to get less than it needs while another gets an overdose. An overdosed animal or bird will retain the drug in its tissues after the required withdrawal period has elapsed and the meat and meat food products derived from them will be unwholesome because of residues.

In conclusion, when the veterinary practitioner makes a decision on whether the affected animals or birds should be given a course of treatment or they should be salvaged through slaughter, he has an additional responsibility, not only to his client, but to the public who will consume the meat and meat byproducts derived from them. When a veterinarian decides to treat or recommends slaughter for human food, it is essential that the public health and aesthetic values of the consumer be considered. Animals and poultry unsuitable for treatment or human food should be disposed of by slaughter on the farm followed by burial, incineration, or processing in a reputable inedible products rendering plant.
REPORT OF COMMITTEE ON 
MEAT AND POULTRY HYGIENE 

73rd Annual Meeting 
United States Animal Health Association 
Milwaukee, Wisconsin 
October 12-17, 1969 

Chairman: W. E. Jennings, Auburn, Alabama 
G. B. Estes, Richmond, Virginia, Co-chairman; E. D. Baker, Madison, 
Wisconsin; A. D. Bond, Chicago, Illinois; L. H. Burkert, St. Paul, Minnesota; 
V. L. Dahl, Columbus, Ohio; W. W. Fechner, Little Rock, Arkansas; M. R. 
Humphrey, Washington, D.C.; D. C. Kelley, Manhattan, Kansas; J. A. Killick, 
Washington, D.C.; J. A. Libby, St. Paul, Minnesota; R. L. Madeira, Elizabeth-
town, Pennsylvania; J. K. Payne, Washington, D.C.; L. J. Rafoth, Chicago, 
Illinois; M. Sonnenberg, Sterling, Colorado; D. H. Spangler, Olympia, Washing-
ton; J. S. Stein, Washington, D.C. 

Your Committee reviewed its report of the 72nd Annual Meeting of the United 
States Animal Health Association, New Orleans, Louisiana, October 6-11, 1968. A 
continuing study of all major topics covered in that report has been conducted 
during the past year. 

Active liaison with the presently established United States Animal Health 
Association's Committees on Mastitis, Public Health and Radiological Fallout, and 
Salmonellosis is being maintained. 

Reports of each of the Subcommittees were reviewed by the full Committee 
and the following was approved: 

I. Information to Appropriate Groups Regarding Requirements of the 
Wholesome Meat and Poultry Inspection Acts: 
Many members of the meat and poultry packing and processing industries 
and consumers are not aware of the requirements of the Wholesome Meat 
and Poultry Inspection Acts though many months have passed since their 
enactment. Therefore, it is recommended that the Public Information 
Office of the Consumer and Marketing Service and the United States 
Agricultural Extension Service be urged to take the following steps to 
increase the flow of information to such groups: 
1. Issue information fact sheets and bulletins to the headquarters of 
state meat packing and poultry processing associations through 
regional Consumer and Marketing Service and State Public Informa-
tion Offices and Agricultural Extension Service Offices. 
2. Hold a conference with Public Information Directors of the national 
and regional meat and poultry processors and associations and representatives of trade publications of affected industries, to 
discover additional avenues for increasing the flow of information 
on these Acts and their significance to industry and to the 
consuming public.
II. 12th Annual Seminar for Teachers of Food Hygiene:

The United States Department of Agriculture's Consumer and Marketing Service is again sponsoring the Annual Food Hygiene Seminar for Teachers of Food Hygiene in the Colleges of Veterinary Medicine in the United States and Canada. This 12th Annual Seminar will be held in Philadelphia on November 11, 12, & 13, 1969.

The theme for the first day's program is "Post Graduate and Continuing Education in Food Hygiene". The second day's theme is, "The Role of the Veterinary College in the Development of Leadership in a National Food Hygiene Program". The third day is devoted to a seminar for preparation of appropriate recommendations on course content of food hygiene courses and other subjects pertinent to instruction in food hygiene in the Colleges of Veterinary Medicine.

III. Post Doctoral Training of Veterinarians in Food Hygiene:

Your Committee reiterates its recommendation of 1968 for Colleges of Veterinary Medicine to provide post graduate training for veterinarians in food hygiene and administration. Your Committee also recommends that the administration of state and federal food inspection programs make veterinarians available for this training as part of a long range employee development program.

IV. Instruction in Food Hygiene in Colleges of Veterinary Medicine:

Your Committee notes with much concern the de-emphasis currently being placed on the instruction in food hygiene in some of the Colleges of Veterinary Medicine in the United States. The expansion of food inspection activities generated by the enactment of the Wholesome Meat and Wholesome Poultry Products Acts necessitates even greater emphasis on this subject in the Veterinary Professional Curriculum than has been given in the past.

Your Committee recommends that the Council on Education of the American Veterinary Medical Association require that the principles of food hygiene be emphasized to all students enrolled in the Veterinary Professional Course in all accredited Colleges of Veterinary Medicine. A resolution on this subject has been prepared and submitted to the Executive Committee of the United States Animal Health Association.

V. Expansion and Coordination of Training for Meat and Poultry Inspection Personnel:

During the fiscal year 1969, the Consumer Protection Program Training Group of the Consumer and Marketing Service provided training for 1850 food (meat and poultry) inspectors and veterinarians. Of these, 636 were employed by state programs. This included advanced training for the meat inspectors at The Ohio State University as well as the initial training of food inspection and veterinarians carried on by the 5 Consumer Protection Program Training Centers. These centers are located at St. Paul, Omaha, Los Angeles, Fort Worth, and Gainsville, Georgia.
Your Committee recommends that the University-based training for food inspectors be expanded to other universities and to provide a wider variety of training courses in the technical aspects of meat production and inspection procedures. Your Committee also recommends the implementation of University-based training for veterinarians employed by meat and food inspection programs. These expansions should be closely coordinated with the upgrading of training available at the five Consumer Protection Program Training Centers and/or state training centers so as to provide comprehensive training programs to better fulfill the needs of inspection personnel.

VI. Intermittent Employment of Students, in Food Hygiene:
Your Committee recommends that the practice of intermittent employment of veterinary students by state and federal meat and poultry inspection services be expanded. This will provide training for future employees who will take part in Federal and State inspection programs.

VII. Topics on Food Hygiene and Inspection in Programs of Veterinary Associations:
Involvement of the total veterinary profession in meat and poultry hygiene is essential for the implementation of new, revised, and expanded programs, resulting from the enactment of the Wholesome Meat and Wholesome Poultry Products Acts of 1967 and 1968 respectively and the meat inspection laws in existence at that time or enacted subsequent thereto in the various states. Your Committee strongly urges each local, state, regional and national veterinary association to include papers and other presentations on food inspection and hygiene in the programs of each of their meetings. This will assist materially in keeping the veterinary profession apprised of developments in the inspection program and, thereby, insure the full support of the total veterinary profession in meeting the challenge which these new inspection laws have presented.

VIII. Food Poisoning Investigation Group:
A cooperative, coordinated program for investigation of the preparation of products derived from meat and poultry when suspected in food poisoning or involved in diseases transmissible to humans, is available through the Toxicology Group, Technical Services Division, Consumer and Marketing Service, United States Department of Agriculture, Washington, D.C. 20250. Your Committee recommends the cooperation with and the use of this group by agencies involved in the investigation of such incidents. For the convenience of these agencies, the telephone number is 301-345-6888.

IX. Interstate Shipment of Meats from State Inspected Plants:
Under the provisions of the Wholesome Meat Act of 1967, product produced in state-inspected plants is not permitted to be shipped interstate. Your Committee feels that this is an injustice to those plants which are
operating under a state inspection system which has been certified as “equal to” federal inspection.

A resolution recommending that the Wholesome Meat Act be amended to permit such movement of product has been prepared and submitted by your Committee to the Executive Committee of the United States Animal Health Association.

X. Cooperative State-Federal Cross-Servicing Laboratories for Meat and Poultry Inspection:

The Committee on Meat and Poultry Hygiene of the United States Animal Health Association in its consideration of this proposal recommends that cooperative effort be made to explore all aspects for establishing a working policy for cross-servicing for each type of laboratory service needed to carry out the regulation requirements under the Wholesome Meat and Wholesome Poultry Products Acts.

In addition, there should be a standardized fee list published for each such service.

XI. Trichinae Eradication Program:

Your Committee has followed with interest the efforts of the swine industry, the packing industry, and the United States Department of Agriculture in developing the pooled-sample, artificial digestion technique for trichinae detection. This test, which was reported on October 16, 1969, by Dr. John S. Andrews and will be described in detail elsewhere in the Proceedings of this 73rd Annual Meeting of the United States Animal Health Association, has proved to be highly accurate and practical. A Program Planning and Budget Model which is based on this test, and combines a consumer protection plan with a national eradication plan, has been prepared for presentation to the Secretary of Agriculture.

A resolution in support of this program has been prepared and submitted by your Committee to the Executive Committee of the United States Animal Health Association.

XII. Coordinated Efforts to Bring Together Needs and Resources for Research in Meat and Poultry Hygiene:

Your Committee recommends that a coordinated effort be made to provide technical information necessary to improve both the efficiency and quality of state and federal meat and poultry inspection programs. Such an effort could result in the orderly application of resources to solving operational problems common to many inspection programs. A standing sub-committee of the Committee on Meat and Poultry Hygiene will be appointed to serve as a medium for identifying problems, setting priorities, locating problem-solving resources (funds, facilities, personnel, etc.), and disseminating the findings.

XIII. Position Statement for School Lunch Procurement:

In The interest of health and physical well-being of this nation's
school children, your Committee recommends that all school lunch program poultry and meat products, whether procured under the Commodity Distribution Program or on a local level under school lunch program general sponsorship, be required to originate only from approved federal, state or state-approved local-inspected plants, and bear the official inspection legend of the appropriate agency.

XIV. Reimbursement to States for Services in Plants under the Talmadge-Aiken Act.

It is the policy of the United States Department of Agriculture to reimburse states on a 50-50 basis for inspection performed by the states in plants operating under the Talmadge-Aiken Act. This is inequitable inasmuch as the states are performing a federal function and are not being adequately reimbursed for this service.

A resolution requesting the Secretary of Agriculture to change the schedule of reimbursement to insure adequate reimbursement to states providing this service, has been prepared and submitted to the Executive Committee of the United States Animal Health Association.

XV. Disposition of Meat from Mature Male Swine:

Meat from mature male swine often exhibits a characteristic odor that is objectionable to many American consumers, although such meat is not considered a public health hazard. In fact, tests conducted by several groups, including the United States Department of Agriculture, universities, foreign health officials and others, consistently show that the meat of more than half of the mature boars contains sexual odor to a significant degree.

Research studies recently completed at Michigan State University and the University of Minnesota demonstrated that meat with sexual odor is likely to be rejected by consumers unless utilized in products in which the odors are reduced in intensity or masked by means of the method of processing, spicing, or dilution. Meat articles found most acceptable for inclusion of boar meat with sexual odor are those that are not usually reheated for use in the home.

Present federal regulations provide for condemnation of swine detected to have pronounced sexual odor. Swine with less than pronounced sexual odor may be passed for use in cooked, comminuted product. However, most mature, male swine are now slaughtered in plants that specialize in boar slaughter. In such plants, boar odor is prevalent at all times. Except in pronounced cases, it is difficult for the inspector to determine the degree of odor present in carcasses by any practical test available to the routine inspector. Few boars are condemned or diverted to processing into cooked, comminuted articles. In fiscal year 1969, less than 1000 boars were condemned in federally inspected plants out of approximately 1,000,000 boars slaughtered during that period. The consumer acceptance problem continues unabated.

To assist in dealing with the disposition of boar meat with sexual odor, your Committee recommends (1) Continuation of present federal
regulations that require condemnation of all swine showing pronounced sexual odor and, (2) Issuance of new regulations to require that meat of all mature male swine not condemned because of pronounced sexual odor, or otherwise, be restricted to use in meat products determined by appropriate testing to be acceptable for inclusion of such an ingredient without the likelihood of objections from the consumer.

XVI. Cold Skinning of Calves and Lambs:

The practice of "cold skinning" of calves and lambs often results in contamination of other carcasses and products with hair, scurf, wool, and manure from hide-on carcasses. The detection and elimination of such unwholesome conditions as injection sites, bruises, grub infestations and attendant damage to underlying tissues poses a serious problem in consumer protection. Your Committee recommends that regulations governing dressing procedures be revised to require that all calves and lambs be completely skinned at the time of slaughter.

XVII. Proposed Federal Regulations:

Many states have a vital interest in the proposed Federal Meat Inspection Regulations published in the Federal Register dated August 14, 1969. Therefore, your Committee recommends that each state make its comments—favorable or unfavorable—directly to the Office of the Hearing Clerk, United States Department of Agriculture, Washington, D.C. 20250, by not later than December 1, 1969.

XVIII. State-Federal Reporting System:

The efficient conduct of an inspection program requires a reporting system to complement the control, direction, and planning efforts of a total inspection program.

Your Committee has concluded it is reasonable to expect that under the cooperative inspection effort provided by the Wholesome Meat and Poultry Products Acts, the participating programs should report their activities by using uniform methods of reporting.

The United States Department of Agriculture and the states are faced with the difficult task of integrating into the Federal Inspection Program Report System, the large volume of data being accumulated by the state programs.

Your Committee recommends that appropriate review and study be undertaken to determining data that should be included in developing a National Inspection Report System. This data should be identified relative to management, operational and budgetary functions. Subsequent to this determination, your Committee urges that individual states and the U.S.D.A. examine their reporting systems to ascertain, first of all, any lesser or greater needs. Secondly, consideration should be given to the adaptability of existing Federal or state forms to satisfy both national and state requirements.
XIX. *The Wisconsin Plan — A New Concept in State-Federal Cooperative Inspection:*

Your Committee took cognizance of the Wisconsin Plan, which is unique among the consumer protection inspection programs of the several states in that it embodies an integrated state and federal effort.

This concept and program, which is a pilot project for state-federal inspection programs, was inaugurated in March 1969. Under Phase I of this program, there is integration of the offices of top state administration and the federal official-in-charge of the federal program within Wisconsin. Phase I also provides for a joint clerical, administrative support, and investigative staff. Coinciding subdivisions of state areas and federal circuits are merged into integrated units with joint clerical staffs. It is anticipated that this will result in improved efficiency of inspection and in substantial overall savings through the elimination of dual facilities, equipment, and personnel. In this phase, the two systems continue to function independently at operating level; however, there is provision for cross-utilization of inspectors in situations that may be geographically duplicative. This, it is anticipated, may result in substantial savings in both personnel and travel expense.

Phase II, when implemented, will find both state and federal systems operating under one administrator, with substantially the same organization at the area-circuit level as in Phase I.

Phase III, which will require some changes in the basic statutes — The Wholesome Meat and Poultry Products Acts — will embody a fully integrated single system with complete cross-utilization and authority of inspectors and supervisory and administrative personnel. This phase will not come about until such time as these changes have been effected. One of the changes considered mandatory for the successful implementation of this Phase, is authorization for certified "equal to" state plants, to ship product interstate.

Your Committee feels that the state of Wisconsin and the Consumer and Marketing Service of the United States Department of Agriculture are to be commended for undertaking this pilot program. Further, your Committee feels that there is considerable merit in this concept and the aspirations of this cooperative effort. A continuing evaluation of this program will be made by your Committee.

XX. *Distribution of Committee Reports:*

In the interest of gaining the greatest results from the recommendations of the Committee on Meat and Poultry Hygiene of the United States Animal Health Association, it is urged that copies of the Committee's report be distributed as needed to the following, immediately upon approval by the Executive Committee of the United States Animal Health Association:

1. The National Food Inspection Advisory Committee.
2. Appropriate Congressional Committees.
3. State Departments and Agency Directors having meat and poultry inspection responsibilities.
4. The American Veterinary Medical Association.
5. Industry representatives and industry related organizations including trade publications.
6. Secretary of Agriculture.
7. Colleges of Veterinary Medicine.
REPORT OF THE COMMITTEE ON
LIVESTOCK MARKETS AND TRANSPORTATION

Chairman: G. C. Stiles, Jefferson City, Mo.
L. H. Butler, Jr., Phoenix, Ariz.; J. H. Brashear, Oklahoma City, Okla.; F. S.
Lee, Brownlee, Nebr.; J. N. McDuffie, Atlanta, Ga.; M. D. Mitchell, Pierre, S.
Dak.; E. E. Montgomery, Washington, D.C.; F. W. Peterson, Omaha, Nebr.; A.
G. Pickett, Topeka, Kans.; C. T. Sanders, Kansas City, Mo.; A. P. Schneider,
Boise, Idaho.; R. Schnell, Dickinson, N. Dak.; F. J. Schoenfeld, Salt Lake City,
Utah; D. H. Spangler, Olympia, Washington; Ingvard Svarre, Sidney, Mont.

The problems of livestock markets and transportation relating to disease
control and eradication or Animal Health are still many and varied and have not
changed much since this Committee met last year. Some of these problems are:
1. There are entirely too many different classifications of markets such as
   a. Specifically approved under Part 78.
   b. Specifically approved under Part 76.
   c. Federally inspected stockyards
   d. Terminal markets
   e. State inspected markets
   f. Livestock auction markets

   We have reached a period of time in our methods of marketing livestock in
which there are no appreciable differences in the manner in which animals are
handled or marketed in any of these so called different classes of markets.
Therefore, it would be reasonable to drop all these different classifications and
simply say that a livestock market is a livestock market. All markets should receive
the same type of inspection and have the same requirements as to facilities. This
then would do away with all the specific approvals and confusion that exists
concerning the markets.

2. Because of these different classifications, the quality or degree of
inspection is different and is geared to fit certain diseases or disease
eradication programs. That is to say that much emphasis is placed on one
or two diseases with little or no regard for any of the other many diseases
that are being spread daily over the nation.

3. Many laws and regulations focus on good, adequate facilities to aid on
control of disease spread, but do not provide for adequate or proper
inspection for disease itself. Facilities alone will not prevent the spread of
disease. There is a great need for uniform and systematic procedures to be
used in handling all diseases detected in livestock in transit regardless of
what market or in what state it might be found.

4. Many markets do not have complete inspection yet they enjoy the same
privileges that completely inspected markets have. That is to say that only
certain classes of livestock are inspected or tested with little or no
attention given to any other classes in the market. Markets should be
inspected in accordance with classes of livestock and all diseases associated
with the different classes of livestock and not just for one or two specific
diseases.
5. There is some disease being spread by livestock that do not pass through markets. More attention is needed in the area of direct movements of animals and of animals through concentration points that are not considered markets.

After much discussion of these problems, the Committee again makes the same recommendations that were made last year dealing with the need for uniform inspection and releasing of livestock from all markets and again the Committee requests the USAHA to urge the USDA to implement last year's recommendations which are as follows:

“For animal health purposes, a single set of standards covering all species, for livestock markets be developed and the inspection of such markets be a joint cooperative state-federal effort, and that prior to release all livestock meet the interstate and state of destination requirements.

The above can best be handled by a Memorandum of Understanding between the State and Federal Animal Health officials and the livestock market management in the respective states. If the above recommendations are carried out, it will replace the need for specifically approved markets, and will place all livestock markets meeting the standards on an equal basis.”

The definition of a Livestock Market and a Livestock Dealer and the Animal Health Standards relating to the facilities used in conducting a livestock market business that was recommended by this Committee last year have not been changed and are again a part of this year's recommendations.
REPORT OF THE
STATE-FEDERAL RELATIONS COMMITTEE

United States Animal Health Association
Washington, D.C.
February 10-14, 1969

Chairman: Frank B. Wheeler, Baton Rouge, Louisiana

Animal Health Division

This Committee will not comment on all program areas, but will emphasize areas requiring special attention.

The cutback in baggage and cargo inspection has increased the potential hazard of introduction of plant, animal diseases, and pests to a dangerous level. Present surveillance levels is not statistically sound and is not applicable to problems involving biological phenomena. There is an urgent need for research to develop a more modern, thorough, sophisticated system of inspection or treatment to insure the safety of Agriculture.

The Committee suggests one way of funding such a project would be to assess the travelers entering the United States. Last year 202,000,000 people entered our Country and an assessment of $1 each would provide adequate funding to start a research program and increase our surveillance.

This Committee supports the efforts of the ANH to make more efficient use of their veterinary resources. By more careful selection of personnel for further technical and professional training and assignment to more responsible positions on the basis of capability and not on the number of personnel supervised.

Based on the experience during the past season in which adequate funds were not provided to support the present demands of the screwworm program, we recommend that the budget base for this program be increased by $750,000. A minimum contingency fund of $1,000,000 be provided so when ideal fly breeding conditions create an emergency situation in the barrier zone, sterile fly production could be increased to cope with this problem. Consideration should be given to forcing the barrier zone from the existing Texas, Mexico border to the Tehuantepac region of Mexico. While initial annual expenditures would be greater than the present, the overall cost would be amortized in eleven and one half years. A long range savings in excess of 50% would result.

We endorse the proposed changes in hog cholera vaccine regulations. An additional $300,000 should be provided to assure full indemnification for swine destroyed in this program.

We urge the development of a workable control and eradication for trichina. The brucellosis eradication efforts in non bovine species should be expanded. Support should be given to the development of the card test for diagnosis of anaplasmosis and to the proposed pilot project to ascertain the safety and efficacy
of the new attenuated vaccine.

United States Animal Health Association believes that ANH working with AVMA and the National Mastitis Council should develop a practical realistic program for the effective control of Mastitis. This should include the establishment of meaningful uniform laboratory procedures and standards on a national basis. The Division should work cooperatively with state regulatory agencies in this endeavor.

It has become evident that Salmonella control cannot be accomplished by survey programs alone. Emphasis must be shifted to specific projects with epidemiology and surveillance at the herd and flock level.

Funds have not been provided for the administration of the Animal Welfare Act. As a result, this activity has been conducted at the expense of other programs. We recommend the immediate funding to adequately conduct this program as required by the Act.

The establishment of a national diagnostic reference laboratory has been suggested by this Committee for several years. Lack of sufficient funds has prevented the ANH from following through on planning and location studies. The need for this facility, working with our state laboratories is urgent.

We solicit support from ARS for the establishment of a pilot project for a national morbidity-mortality reporting system as recommended by the National Academy of Sciences.

Summary:
1. Need for improved methods of inspection at Ports of Entry.
2. More efficient use of veterinary manpower resources through improved training.
3. Consideration of a new barrier zone for screwworm program.
4. Endorsement of changes in hog cholera vaccine regulations.
5. Trichina Eradication Program.
6. Increased effort for Brucellosis eradication in non bovine species.
7. Development of card test and vaccine study for Anaplasmosis.
8. Need for a National Cooperative Mastitis Control Program with uniform standards.
9. Shift in Salmonella emphasis.
11. Need for a National Animal Diagnostic Reference Laboratory.
12. Establishment of a National disease reporting system.

Animal Disease and Parasite Research Division

It is said that if all diseases affecting meat-producing animals could be eliminated, 100 million more people could be fed each year without increasing our livestock population by a single head. If this is true, then research toward this goal is certainly among the most important of our endeavors. Yet, last year many of our pleas for the Animal Disease and Parasite Research Division either went unheeded or were curtailed in favor of other projects. If animal agriculture is to cope with the everworsening food problem, these pleas must not be perennially denied.
Southwestern Veterinary Toxicology and Livestock Insects Research Laboratory, College Station, Texas.

This facility is scheduled for completion around the end of 1969. Seven scientist positions were requested to supplement the present staff of three, now working in temporary quarters. This item was deleted from the 1970 budget. It seems pointless to construct such a facility and then maintain it virtually empty. Certainly the development and safety of the various chemicals upon which animal agriculture is dependent for its survival should occupy a near-top position in national priority.

Beltsville Parasitological Laboratory

Last year, the United States Animal Health Association urged all-out support for the Animal Disease and Parasite Research Division’s effort to bring this facility up to its full potential. However, financial support for the effort was deleted from that budget, and we now learn that this deletion has been repeated this year. Losses from parasitism in domestic animals are presently estimated at $1 billion annually in the United States, and this figure is expected to increase.

Diseases such as anaplasmosis, piroplasmosis, and trichinosis should be investigated to develop knowledge for their eradication from the United States. There is urgent need for the application of disciplines requiring more than average laboratory space, such as pathology, genetics, biochemistry, immunochemistry, and others. The diagnosis, treatment, and biological control of protozoan and helminthic parasitism would be greatly strengthened by artificial cultivation of organisms, study of host-parasite relationships, and the contributions of various disciplines. It has been proposed to locate a facility for this purpose on the premises of the Agricultural Research Center. Establishment of such a laboratory elsewhere would require an amount far in excess of the proposed investment at Beltsville since the subject proposal presumes full utilization of present premises and buildings, which represent an investment of some $13 million. The concentration of scientific knowledge and literature in this area makes the proposed location especially desirable from the standpoint of research effectiveness.

National Animal Disease Laboratory

The United States Animal Health Association is concerned that more rapid progress has not been made in moving the Biologics Division and diagnostic services out of the quarters presently occupied at Ames, Iowa. These services could well use facilities requiring less security, thus releasing this space to the Animal Disease and Parasite Research Division to whose work it is much better suited. There is great need for a pathoecology facility for the study of environmental factors in animal diseases.

Plum Island Animal Disease Laboratory

The Association regretted exceedingly the withdrawal from the 1969 budget of $650,000 requested for additional facilities at Plum Island. Limited facilities have severely impeded progress in the development of information concerning exotic diseases, at least twenty of which are spreading in various parts of the world and even now pose a threat to the United States. In the face of ever-increasing and more
rapid transportation of both people and animals all over the world— and more specifically, to our own shores—the need for knowledge to quickly identify and control exotic diseases assumes an almost frightening urgency. This, coupled with the United States Department of Agriculture’s responsibility for our defense against biological welfare in animals, makes research mandatory for development of the knowhow and the tools essential to our national safety. This is an area where we simply cannot afford “too little, too late.”

**Plum Island Environmental Control**

The Department of Health, Education, and Welfare has advised the Animal Disease and Parasite Research Division that the Plum Island facility does not conform to standards of the National Air Pollution Administration. To avert immediate work stoppage, the Division sought, and was granted, a one-year extension of the order to comply. If this item is deleted from the budget, the Division will be unable to show “intent to comply,” and the facility will be faced with closure on or about October 1, 1969. The Division will need $319,000 for the required modifications.

**Bluetongue—Denver, Colorado**

The knowledge that bluetongue in cattle in the United States was cause for the restriction and rejection of certain livestock exports to Australia and Ireland places a priority on the work on bluetongue scheduled for the Denver Laboratory. The development of additional facilities for research in this area will take $40,000 for planning and approximately $460,000 to construct required facilities. This will permit augmentation of present research efforts at the Denver Laboratory to develop new and improve existing methods for the diagnosis of bluetongue in sheep, cattle, and goats.

Efforts will also be made to determine the reservoirs of infection and the length of time animals may retain carriers of the disease.

Considerable additional effort will be placed on the disease as it relates to cattle.

The additional facility will provide needed space for complete integration of team research involving the Entomology Research Division and the Animal Disease and Parasite Research Division in studies designed to resolve questions concerning complex problems of transmission.

The recognition of the disease in cattle has increased the possibility for an extended range of vectors which may be involved in transmission. Data on vector transmission is essential for consideration in the formulation of control programs where they are limited or aimed at total eradication. The facility will also provide opportunities to study the carrier problem, duration of viremia, and duration of immunity, each of which will have a marked influence on the efforts aimed at the solution of the problem.

Studies should be extended to determine if wild ruminants or other wild animals are reservoirs.

**Veterinary Biologics Division**

The Committee wishes to express appreciation for the cooperation extended it
by the Director and his staff in explaining the aims, goals and requirements of the Veterinary Biologics Division. The testing of Veterinary Biologics continues to show improvement, but a definite lag still exists in the testing for sterility and potency of many products. Last year the Committee pointed out that there is still a critical need to develop proper facilities and to obtain additional trained personnel.

If the Division is ever to reach and maintain a "current status" it becomes imperative that adequate test animal facilities and testing laboratores be acquired. The Committee was advised one year ago of a proposal by Colorado State University to provide the required facilities. This proposal and need still exists, therefore, the Committee urges that immediate steps be instituted to investigate and develop this proposal thereby relieving the inefficient arrangement which exists at NADL, Iowa State University and the surrounding community. The cost of the Colorado State University proposal will represent an ultimate savings to the tax payer.

Reagents and reference products are urgently needed. These must be developed, evaluated, and distributed by the Division. This is the only means by which uniformity and validity of testing by licensed establishments and the Division laboratory can be assured.

The Committee was advised that a preliminary study conducted by the ARS Biometrical Services indicated that present methods and sampling techniques were not statistically valid, therefore, the Committee feels that biometrical assistance should be provided to develop and improve the statistical validity of test methods and sampling.

It is evident to the Committee that worthless, contaminated, and dangerous products are still available for use within our livestock and poultry industries. The Committee feels that proper field investigation of all complaints would serve a useful purpose and that the Division should request sufficient funds to implement a program of this kind.

Safety and efficacy of licensed veterinary biologics is the primary responsibility of the Division. In order to accomplish this responsibility adequate testing methods are required because methods have not been developed. A program for the development of adequate test methods should be implemented. The Committee urges that funds be requested in the next appropriation of sufficient amount to initiate a program of test method development. Many of the test methods now employed need improvement in order to assure product quality. The Committee feels that these two items could be considered at the same time.

Summary:

1. The Committee continues to support the Division's interest in leasing adequate facilities at Colorado State University.

2. The Committee supports the additional appropriation requests, but does not feel it to be adequate in view of other recommendations.

3. The Committee urges the Division to institute adequate biometrical assistance to develop and improve the statistical validity of test methods and sampling.

4. The Committee urges the Division to develop a program of investigation on field complaints.
5. The Committee supports the Division’s need for more and improved test methods to assure safety and efficacy of all veterinary biologics.

**Consumer and Marketing Service**

The United States Animal Health Association appreciates this opportunity to discuss the many problems that exist in those areas where the states have common interest with the Consumer and Marketing Service. Our specific concern, of course, is with the inspection for wholesomeness of Red Meats and Poultry.

The Talmadge-Aiken Act of 1962 authorizes the Secretary to cooperate with the various states in the use of state personnel to provide those services required of the Secretary for product in commerce. This authorization is now used in the area we are here concerned with. Talmadge-Aiken of T.A. agreements have now been approved with 28 of the states, establishing such cooperative program in meat inspection.

The so called Wholesome Meat Act was passed in late 1967. Here again federal law offers the states cooperation in the form of guidance, training, and financial assistance. Participation on the part of the states is still left voluntary as in the T.A. Act. This law specifies, however, that if the states do participate they must bring their inspection services and the industries plants to equality with federal standards within a two year period. Whether or not the states choose to cooperate the Secretary is authorized to step in and “take over”, as it were, if this is not accomplished within the time specified.

Problems have developed from the very start of these two programs. The atmosphere was not conducive to amicable relations. Mistakes have been and are being made by both state and federal personnel. The U.S. Animal Health Association earnestly desires to provide the forum where the two agencies – state and federal – may meet and resolve their differences, and settle their mutual problems, not only amicably but to the satisfaction of each one of the participants. The U.S. Animal Health Association has had more than 70 years experience in doing just that. This experience is now at the service of the U.S. Consumer and Marketing Service, and we urge that it be fully and freely used.

We have no desire here to deal at any length with the specifics of our mutual problems. Some of our problems are major ones – many are minor and petty but are direct outgrowths of the larger issues.

Our communications greatly need improvement. If we can achieve this, hopefully, mutual respect and confidence will be firmly established. The U.S. Animal Health Association has vast experience in this area also.

Clarification of terms, aims, and intent would greatly improve relations and hasten compliance, if not with the letter, with the intent of federal law. Originally we were told that “equal to” meant the same as “identical”. This week the term “meaningful comparability”, was used. Hopefully we see the rule of reason emerging here. So long as the public interest is not placed in jeopardy, we have long felt that, “meaningful comparability”, would fulfill the intent of Congress and is achievable within a reasonable time.

We must cooperate. The public interest surely demands it and the Congress has recognized this need. Perhaps we need to re-define the term, but with respect, we would remind both parties that Domination is not Cooperation. Leadership is,
certainly, along with advice, guidance, and assistance — but not domination. There are many in this effort who do not understand this as yet.

The term, "meaningful cooperation", was also used this week. Here again we are encouraged. Let us be full partners in providing a wholesome meat supply to all of our citizens.

We appreciate the interest of the Consumer and Marketing Service in our organization. It was gratifying to be able to provide them with a seat on our Executive Committee. This avenue of communication should provide valuable indeed. The recent letter of Dr. Somers to our President requesting representation on several of our standing committees will receive prompt attention.

There are several matters pending, or under consideration, such as using lay inspectors for final inspections of retained carcasses and allowing condemnation without professional decision, the new feeling about the use of beef lungs, or in fact any lung tissue in product, the exportation of inedible fat with the chance of it being returned to this country in edible product, and others, about which we have great and in some cases grave concern.

We would like to openly and freely debate these issues in our Meat and Poultry Hygiene Committee, following which a firm position and recommendation will be made as needed.

The U.S. Animal Health Association meets once each year during October. Many other groups and organizations who share our interest find it both desirable and rewarding to meet with us. We earnestly hope and urge the Consumer and Marketing Service, Meat and Poultry inspection people from here and all over the nation to do the same. We promise you a warm welcome, the hand of friendship, and our full cooperation — in every sense of the word.
BOVINE MASTITIS CONTROL
ITS PRESENT STATUS

S. J. Roberts, D.V.M., M.S.*
A. M. Meek, Ph.D.**
R. P. Natzke, Ph.D.**
R. S. Guthris, D.V.M.*
R. W. Everett, Ph.D.**

Bovine mastitis is the most important uncontrolled infectious disease of dairy cattle in the United States. The yearly economic loss inflicted by bovine mastitis has been carefully estimated to exceed 1 billion dollars, about 70 dollars per cow or $2,800 per herd of 40 milking cows. This figure reflects losses in milk production because of subclinical and clinical infections, treatment costs, losses incurred from culling severely affected cows, and the cost of their replacements. Nevertheless, farmers and veterinarians all too frequently accept these losses as an inevitable part of the cost of milk production. In addition to economic loss, udder infections usually cause lowered milk quality due to increased leucocyte levels of the milk in subclinically, as well as clinically, infected quarters. Extensive surveys have shown that about one-half of all dairy cows are infected in one-half of their quarters. Thus mastitis is a herd problem not an individual cow problem and it must be treated on a herd basis of control is to be achieved.

Within the last year four excellent review papers on mastitis control have been published1,3,4,6. This paper is an amalgamation of the control measures for mastitis presented by these authors followed by a brief preliminary report on a large field trial of a simple mastitis control plan being conducted in New York State. In the past most studies and research on mastitis were small experiments conducted on relatively few animals. From these numerous limited studies the many complex facets of mastitis were elicited but attempts to organize these many aspects of knowledge concerning mastitis into a fairly simple, practical control program appeared insurmountable. Therefore, many scientists lacking adequate support for large, carefully controlled field trials turned away from mastitis research to more narrow and productive fields of study. In recent years the importance of mastitis control, the advent of group or cooperative approaches to large experimental studies and the introduction of electronic data processing procedures has stimulated a few large, controlled field studies on the eradication of mastitis infections, sanitation practices, therapeutic compounds and milking machine factors which are known to be components of the mastitis complex.

*Mastitis infections. It has been established that nearly all of the 20 different types of pathogenic organisms isolated from infected udders enter the udder through the teat orifice and streak canal. Of these 20 agents, 4 gram-positive bacteria, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Staphylococcus aureus*, cause 97% of all udder infection. Gram-negative bacilli such as *Escherichia coli*, that

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abound in the external environment of the cow's udder, cause less than 1% of udder infections. The incidence of bovine infectious in New York was determined from cultures of milk samples taken from 242,871 cows in 7,320 initially surveyed herds over a period of 20 years (1946 through 1965). Initial samplings indicated infection rates of 27% *Streptococcus agalactiae*, 18% streptococci other than *Streptococcus Agalactiae*, 13% staphylococci, and 1% bacilli; 41% of the cows were free of infection. Approximately 75% of the infections were subclinical, not detectable by palpation of the gland or strip-cup examination of the udder secretion, 24% had mild to moderate signs of clinical mastitis, and 1% has severe clinical mastitis characterized by swelling of the gland and often general illness. Very high leucocyte numbers are present in moderate and severe clinical mastitis. Subclinical infections, especially with *Streptococcus agalactiae*, are also characterized by the shedding of large numbers of leucocytes detectable only by a screening test or direct microscopic cell count. It is obvious from past studies that these various forms of mastitis each have different epidemiological and pathogenic patterns of udder infection. The teat canal with its keratinous lining is largely responsible for preventing quarter infections from organisms contaminating the external skin and orifice of the teat.

Udder and teat skin contamination or infection may spread from cow to cow or quarter to quarter at the time of milking by means of the milker's hands, contaminated wash cloths or sponges and by teat cups. Infection may spread at other times by contaminated bedding, suckling calves or cows, flies and by switching of tails especially in stanchion barns. Despite common opinion recent studies have conclusively shown that udder infections gain entrance to the gland between milkings during the lactation period and during the nonlactating or dry period. Infections seldom occur during the milking act.

Once a quarter becomes infected a number of events may occur. Both subclinically infected cows and cows with acute mastitis may recover spontaneously or after appropriate antibiotic therapy. Death may occasionally result from severe mastitis. Untreated infected quarters may progress from subclinical to clinical infections and then regress to the subclinical form again. Once a quarter becomes infected, it often remains chronically infected for months or years and during this period the signs of infection may occasionally exacerbate. Claims are often made that a recommended change in management or hygiene immediately corrected the problem of udder disease in a herd. This may be true of clinical mastitis, but it is clearly impossible with respect to subclinical infections.

Sanitation of hygienic practices commonly recommended to control mastitis include daily washing and sterilization of milking equipment, use of individual sterile towels, use of a mild disinfectant for washing the udder and teats before milking, rinsing and dipping teat cups first in water and then in a disinfectant solution between cows, dipping teats in a milk antiseptic solution after milking, providing adequate bedding to keep cows clean and dry, and eliminating muddy, filthy barnyards.

There is much disagreement on the individual or collective value of the present hygienic practices for preventing the spread of udder pathogens. The commonly recommended milk udder disinfectants, even if properly used, may reduce but do not prevent the spread of these infections. Under ideal, strict, but impractical, sanitary conditions, the spread of streptococci and staphylococci from cow to cow...
can effectively be prevented. While not preventing the spread of pathogens, dipping of teats after milking is one sanitary practice that can greatly reduce organisms on the teat skin and orifice.

English studies have shown and American experiments have confirmed that dipping of teats immediately after milking in an effective disinfectant is the most important single hygienic measure for controlling the spread of udder infections. Proven effective disinfectants for teat dipping are 4 to 5% (40-50,000 p.p.m.) sodium hypochlorite solutions with less than 9.08% sodium hydroxide content(1) and a 1.0% (10,000 p.p.m.) neutral iodophor preparation(2). Although the English workers questioned its effectiveness, McDonald (1968) reported the 1% chlorhexidine solution containing 6 oz. of glycerine/gallon(3) was an efficient teat dip. It should be noted that teat ointments containing phenol should not be used on cows whose teats are dipped in sodium hypochlorite or an objectionable medicinal odor may form and taint the milk.

*Therapeutic or antibiotic compounds* have been widely used by intramammary infusion by farmers and veterinarians for the past 20 years for the treatment of chronic or acute mastitis. These products have varied greatly in their efficacy against the various forms of udder infections. The common practice of infusing antibiotic preparations of questionable value in an indiscriminate manner does not constitute a satisfactory mastitis control program. The present control programs followed in states like New York and Connecticut consist of surveying herds to obtain bacteriologic data on each quarter of each cow, together with immediate and appropriate treatment of infected cows. These programs have proven highly effective in those herds with severe mastitis problems and in those where the owner is trying to eliminate *Sta. agalactiae*. However, this procedure is rather costly and time consuming. Its value in the past has been reduced by the lack of proven hygienic procedures to prevent on control new infections. Therapy during the lactation period based on clinical or screening tests is of very limited value in controlling herd mastitis infections. It is costly due to the discard of milk even when based on cultural tests.

Although some veterinarians and farmers have been treating cows during the dry period for many years, until recently no antibiotic compounds designed for the treatment of nonlactating cows have been developed. In past years any benefit gained in the reduction of infection by therapy during the dry period was promptly nullified by the rapid development of new infections during the lactation period because of the lack of a satisfactory hygiene program. The routine treatment of all quarters in cows at the end of lactation with an effective antibiotic preparation would ensure treatment and a high level of elimination of all infections not detected and treated during lactation. Treatment during the nonlactating period would also reduce the problem of antibiotic contamination of the milk supply. Because the preparation is not milked out and because a more potent antibiotic

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1. *Clorox*, a 5.25% sodium hypochlorite solution with a sodium hydroxide content of 0.01% that may be diluted one-quarter with water, Clorox Company, Oakland, Calif.
2. *Bovadine* (Vetadine) a 1% iodophor preparation, Lazarus Laboratories, Inc., a division of West Chemical Products Inc., Long Island City, N.Y.
3. *Novalsan*, 1% chlorhexidine, Fort Dodge Lab., Fort Dodge, Iowa.
Figure 1.

PROGRESS ON ELIMINATION OF SPECIFIC FORMS* OF MASTITIS INFECTION IN 25 DAIRY HERDS

<table>
<thead>
<tr>
<th>Duration of Experiment</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus agalactiae</th>
<th>Streptococcus uberis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Herds Free</td>
<td>Herds Low Level</td>
<td>Herds Infected</td>
</tr>
<tr>
<td>State</td>
<td>0</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>12 months</td>
<td>0</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>24 months***</td>
<td>2</td>
<td>15</td>
<td>8</td>
</tr>
</tbody>
</table>

* At the start of the experiment 88% of 2,584 quarter infections were *Staph. aureus* (41%), *Strep. agal.* (30%) and *Strep. uberis* (17%).

** Herds low level = less than 5 infected quarters in the herd; infected herds had greater number of infected quarters.

*** At 24 months, 237 quarter infections of these 3 forms were present in the 25 herds compared to 2,264 quarter infections at the start of the experiment.
preparation in a slow-release base could be used, greater elimination of udder infection could be expected. Also, therapy at this time would reduce the development of new infections in nonlactating cows by maintaining prolonged antibiotic concentrations in the secretion the first critical weeks and throughout most of the nonlactating period. Furthermore costly surveys or cultures of all quarters in the herd is not essential or necessary. This practice of dry cow therapy has greatly reduced the problem of cows freshening with mastitis with a resulting severe drop in milk production for that lactation period.

Although the English coworkers developed a benzathine cloxacillin with aluminum monostearate preparation and the Cornell experiment paralleling theirs has used an experimental high dosage penicillin and streptomycin with aluminum monostearate preparation, no proven, effective dry cow treatment compounds are presently available commercially in the United States. It is hoped that the latter preparation will soon be approved by the F.D.A. and that other proven products for dry cow therapy will follow.

_Fmilking machine factors_ causing the development of clinical mastitis from subclinical udder infections are considered of secondary important in the control of udder infection. There is no good evidence that the make or type of milking machine, the vacuum level, or the state of repair of the machines influence the incidence or spread of udder infection in lactating cows. McDonald (1969) has reviewed the effect of various milking machine factors on mastitis. Although he cited limited studies that reported increased intramammary infections associated with fluctuating vacuum, a high pulsation rate and a wide pulsation ratio, further work is obviously needed. Noninfected quarters or udders may be severely abused by defective machines without clinical mastitis being produced.

_Other methods for controlling mastitis_ include immunization and selection for genetic resistance. Although numerous attempts have been made to prevent udder infection by immunization the multiple strains of the many causative mastitis organisms have complicated and limited this means of control. The use of commercial staphylococcal vaccines have been of questionable value. Some reports have indicated that autogenous staphylococcic vaccines may reduce the incidence of new infections ad well as the severity and duration of clinical signs of staphylococcic mastitis in a herd. Streptococcal vaccines have been produced experimentally but require controlled large scale field testing, Norcross. Controlling udder infection by selective breeding of resistant cattle might be possible. However, progress would likely be slow because criteria on which a selection program could be based have not been determined and other inherited traits might well be considered more important.

_The Cornell experiment_ — Preliminary results on a three-year experiment with a simple mastitis control program modeled after one being conducted in England of teat dipping with a 4% sodium hypochlorite solution after each milking and nonlactating cow treatment of each quarter of every cow after the last milking with 1 million units of penicillin and 1 gm. of dihydrostreptomycin with 3% aluminum monostearate in peanut oil have shown that after two years the level of quarter infection of about 2,000 cows in 25 Holstein herds in New York State(6) dropped from 40% to 8.4%. The dramatic reduction in quarters infected with streptococci and staphylococci can be noted in Figure 1. This experiment indicates that _Staph._
aureus, Str. agalactiae and Str. uberis, responsible for 88% of the initially infected quarters, can be largely eliminated from dairy herds. Clinical infections have been reduced but not to the extent that would be indicated by the decline in the level of infection. Recent clinical infections are those caused largely by Str. uberis, E. Coli and entero-cocci. This needs further study. Str. agalactiae has been eliminated in a majority of the herds without resorting to repeated surveys and lactation therapy that have been standard procedures in the Str. agalactiae eradication programs followed by New York State and Connecticut.

Although more large, controlled field experiments and smaller detailed studies on mastitis will be needed to refine the control procedures for this disease, we now have for the first time a simple, cheap, practical control program that can be instituted by any veterinarian or dairyman. It appears from the results of the English and American experiments that if this program is followed carefully and continuously it will largely control the major ravages of mastitis, produce a good quality product, increase the production of milk per cow and raise the farmer's income.

REFERENCES


REPORT OF THE U.S.A.H.A. MASTITIS COMMITTEE
1969

Chairman: K. J. Peterson, Corvallis, Oreg.

The U.S.A.H.A. Mastitis Committee in its 1968 committee report recommended that a meeting with the A.V.M.A. Mastitis Committee and representatives of the National Mastitis Council be arranged. This was accomplished and the three groups met in Chicago January 28, 1969, during the annual meeting of the National Mastitis Council. Dr. K. J. Peterson, chairman of the U.S.A.H.A. Mastitis Committee, presided. This was the first joint meeting of representatives of U.S.A.H.A., A.V.M.A., and N.M.C., the purpose of which was to develop a more complete understanding of the functions, achievements, and objectives of the three organizations and to coordinate programs in bovine mastitis abatement. A lengthy discussion of the various aspects of mastitis control followed. This included treatment, milking machines, sanitation, *Streptococcus agalactiae* eradication, and interpretation and use of indirect mastitis test results. It was agreed that cooperative efforts of the U.S.A.H.A., A.V.M.A., and N.M.C. are necessary in solving the complex problems of bovine mastitis. It was further agreed that a meeting of representatives of these three organizations be held annually. The next joint meeting is tentatively scheduled for the day prior to the 1970 N.M.C. meeting in Chicago.

The U.S.A.H.A. Mastitis Committee held its annual open meeting October 13, 1969 in Milwaukee. Eight committee members and 25 interested guests attended.

A paper entitled “Mastitis Testing from a Statistician’s Viewpoint” was presented by Mr. Alen Sturges of the Wisconsin Department of Agriculture.

A lengthy discussion concerning development of a national program aimed at the control of mastitis caused by *Streptococcus agalactiae* followed. The committee accepted a motion that a sub-committee be appointed by the chairman to develop methods and means of conducting a *Streptococcus agalactiae* survey to determine the herd incidence of this disease within several states representing the various geographical regions of the United States.

Considerable discussion developed over the desirability of establishing standardized procedures for defining *Streptococcus agalactiae* free herds. However, no definite action was taken.

The committee again recommended that the U.S.A.H.A. maintain continuing membership in the National Mastitis Council.

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WHERE DOES THE RESPONSIBILITY LIE?

Dr. R. J. Kirkpatrick

U.S. Animal Health Association
October 14, 1969

At present we have over 200 million people in the United States. It is estimated that we will have 340 million by the year 2000. Add to this a higher growth rate in many countries of the world, plus the fact that we are feeding more of the world. People are hungry today. We are short on protein. U.S.D.A. suggests we will need twice as much meal, milk and eggs within 30 years for our growing population.

Losses from diseases, parasites and insects are stealing an estimated 2.8 billion dollars worth of food each year. Major reductions in these losses has not been evident in the surveys I have reviewed. Where does the responsibility lie to reduce this staggering loss?

I am concerned over this failure of the community represented here today to produce satisfactory or at least desired results. Are we directing our best efforts toward this problem? My concern leads me to surmise that if we do not meet the challenge in an efficient manner we will face public pressure as shown by action in congress or the courts. Often public reaction creates injury or hardship, rather than solutions.

Scientific advancement during the last decade has provided many tools to be used by government, both state and federal, by the academic institutions, by researchers, by the drug and biologic industry, by the veterinary practitioner, and ultimately by the livestock and poultry producers.

Let us examine each of these segments of livestock production and observe their balance sheet.

First the federal government. Uncle Sam, through F.D.A. and A.R.S., is monitoring, regulating and controlling more disease prevention and treatment than ever before. Eradication programs are popular. Congress and the Nader’s of today are clamoring for more action. The public is demanding consumer protection.

Perhaps a few questions can help you evaluate whether the government is satisfying its responsibility:

1. Is there a balance between the extreme emphasis placed on the development of safe and effective products with very exacting labeling and the lack of emphasis placed on the ability of the user to diagnose and know when to use the product?
2. Is proper balance being given to the benefit as well as the risk of useful products, especially in the antimicrobial and hormone area?
3. Is bureaucracy blocking productive activity?

Second, let’s look at state government, and its part in disease prevention and control.

We see more restrictions on animal movement, stricter enforcement and more control over animal health products used. These activities should produce desired results.

But can we ask:
WHERE DOES THE RESPONSIBILITY LIE?

1. Are personnel adequate to do the job?
2. Are salaries high enough to keep good people?
3. Does agriculture appreciate and cooperate with your service?
4. Are livestock concentration points properly regulated so that health inspection or certification is meaningful? Do you give the veterinarians a fair chance or is their position untenable?

Next, let's look to the colleges — primarily the colleges of Veterinary Medicine. Budgets are bigger, staffs are more adequate and better trained. Students are tops. Extension departments are well organized. Research activity is being featured. It would appear the colleges are discharging their responsibility.

However:
1. Is the graduate prepared to serve the agricultural community of today with its varied, specialized interests?
2. Does the curriculum allow for specialization?
3. Are buildings and plants given priority over curriculum or staff?
4. Are they educating the public in health management?
5. Are instructors given time to instruct or must they give a high proportion of their time to grant research so that budgets are met?

The veterinary profession has a direct charge to treat and prevent disease. They are well trained in recognizing disease and in therapeutic and preventive measures. Fees are improved. Farms are getting bigger with livestock more concentrated. Drugs are safer and more efficacious than ever before.

Why do we see major losses occurring in each practice area?
1. Is the veterinarian bitching or is he building? Is he changing with the times?
2. Are over-the-fence diagnoses providing the right results?
3. Is continuing education as important as vacations?
4. Is the service available sold to the livestock owner?
5. Has the veterinarian research his market place to know what service is desired?

The drug and biological industry is selling products at an all-time high. The products are well researched and designed to fulfill a need. We market our products well. Where have we failed?
1. Have we convinced the government and the consumer that making a profit is vital rather than dishonest?
2. Do we properly inform our customer as to when to use our product?
3. Have all the "low value" products been deleted from our lines?
4. Are we investing a proper share of profits into better products for tomorrow?

And, finally, the producer. Does responsibility lie with this man? He is better equipped and better educated. Nutrition and genetics have both made the efficient production job easier.
1. Has he kept up with modern discoveries and health management practices?
2. Does he recognize the value of disease experts and health management consultants?
3. Does his record system provide needed alarms for immediate action?
4. Does he buy feeders with no regard to source and health management?
5. Will he change a health management system for ¼ cent a dose?

Where does the responsibility lie? Do each of the groups represented here today have a responsibility to reduce the $2.8 billion loss from disease?

We have much of the know-how, the organization, the manpower, and the power to do a better job in reducing disease losses.

I urge the A.H.A. to design an action program using all groups represented here today. Not only do we need a program designed, but we need to sell and implement the program. If we do not, the hungry lawyers of today will embarrass us all and pocket much of the reward. Or, we'll have a disturbed producer, fed up with the animal health community for failing to meet his needs. He'll naturally demand congressional action.

I have raised many questions regarding priorities; have provided few answers. I know that a mountain cannot be moved overnight, but I am confident that if the talent in this room is harnessed, motivated, and given leadership, results can occur. Where does the responsibility lie?
FURTHER STUDIES ON NEONATAL CALF DIARRHEA VIRUS

Charles A. Mebus, D.V.M., Ph.D.
Norman R. Underdahl, M.S.
Marvin B. Rhodes, M.S.
Marvin J. Twiehaus, D.V.M.
Department of Veterinary Science

Since the report that neonatal calf diarrhea had been reproduced experimentally with a viral agent, immunofluorescent cells have been found associated with this disease in feces from 69 cow-calf herds and 1 dairy herd in Nebraska, 1 cow-calf herd in South Dakota and 1 dairy herd in Illinois. This report deals with confirming the relationship between immunofluorescence and the presence of the virus in feces collected from outbreaks of diarrhea and with determining the similarity of the virus isolates from various herds.

MATERIALS AND METHODS:
Methods of bacteriologic examination, duodenal injection, preparation of conjugate, and the technique for fluorescent antibody staining of sections of intestine and feces have been described. Methods for handling “caught” calves and isolation room procedures also were described. Gnotobiotic calves were delivered by caesarean section in to a previously formaldehyde-gassed surgical isolator attached to a sterile isolator, and were fed autoclaved milk. Tissue culture procedures will be published.

RESULTS:
Whole fecal material containing fluorescing cells collected from a cow-calf herd in Cody, Nebraska, produced diarrhea. On staining with fluorescein labelled antibody, immunofluorescent cells were found to be present in the feces and sections of small intestine from the experimental calves.

In order to compare duodenal injection and oral inoculation one aliquot of a fecal sample from a herd near North Platte containing many fluorescing cells was inoculated into a caught calf (69-3) via duodenal injection. Sixteen hours postinoculation (PI) the calf was very depressed; diarrhea began 17½ hours PI and the calf died 18½ hours PI. Following staining with the calf diarrhea conjugate immunofluorescence was demonstrated in the epithelial cells of sections of upper, middle, and lower small intestine. A bacteria-free fecal filtrate from this calf (69-3) was inoculated orally into a gnotobiotic calf (69-4). Twenty-four hours PI the calf would not suck; 26 hours PI the calf was very depressed but did not develop diarrhea. Forty-eight hours after the initial inoculation the calf was inoculated orally with known infective bacteria-free fecal filtrate of the Cody isolate. The calf remained clinically normal. When removed from the isolation unit, only Bacillus subtilis was isolated from the feces. A bacteria-free fecal filtrate from calf 69-3 inoculated by duodenal injection into a caesarean section-derived calf (69-5) caused a watery, yellow diarrhea 27 hours PI. The calf recovered.

A second aliquot of feces from the North Platte calf was used to inoculate
orally a caught calf (69-6). About 31 hours PI the calf was passing reddish-brown watery feces. The calf soon became moribund, and was killed 35 hours PI. Epithelial-cell immunofluorescence was observed in sections from the upper, middle, and lower small intestine. *Escherichia coli* were isolated from all levels of the small intestine and brain.

Duodenal injection of bacteria-free filtrate of feces from a dairy herd in Illinois that contained many fluorescing cells caused diarrhea 25 hours PI. Epithelial fluorescence was observed in sections of the small intestine.

Duodenal injection of bacteria-free filtrates of intestinal contents which contained no fluorescing cells from a diarrhetic dairy calf (69-23) obtained in Lincoln caused diarrhea in two experimental calves. Oral inoculation of a filtrate of this same material (69-23) in another calf caused severe depression but no diarrhea. Oral inoculation of a gnotobiotic calf with immunofluorescent positive 13th tissue culture passage of this same isolate caused depression about 20 hours PI and diarrhea about 26 hours PI. Forty-six hours after the first inoculation the calf was inoculated orally with a known infective fecal filtrate of the Code isolate; the calf remained clinically normal. When removed from the isolation unit only gram positive coci (staphylococcus) were present in the feces.

**DISCUSSION:**

Confirmation of the relationship of fluorescent cells in the feces to infectivity for experimental calves was obtained following inoculation via duodenal injection of unfiltered or bacteria-free filtrates of feces from herds in Cody and North Platte, Nebraska and Illinois. The experimental calves became depressed, developed diarrhea, and had fluorescing cells in their feces and frozen sections of small intestine.

Duodenal injection was found to be more effective than oral inoculation for reproducing diarrhea when the feces used in the inoculum contained few or no fluorescing cells. Use of bacteria-free filtrate of a field sample as an inoculum was desirable, because when unfiltered material was inoculated the calf frequently died about the time diarrhea should have started or shortly after onset of diarrhea. When this happened adequate material of high viral titer could not be collected.

Antigenic similarity of the viruses causing calf diarrhea was demonstrated in cow-calf herds from Cody and North Platte, Nebraska, and in dairy herds from Illinois and Lincoln, Nebraska. Immunofluorescent cells were observed following staining with the same conjugate in the original fecal specimens and in feces and/or sections of the small intestine from experimental calves. Also, calves recovered from illness caused by the virus in calf feces from North Platte or Lincoln were resistant when inoculated with virus from the Cody herd.

On the basis of the above results, it is believed that the 71 herds in which immunofluorescent cells were demonstrated in the feces of calves with neonatal diarrhea were all infected with the same virus.
REFERENCE

REPORT OF THE COMMITTEE ON
INFECTIONOUS DISEASES OF CATTLE

One of the major voids in regulatory animal disease control in the United States has existed in the artificial insemination industry. This industry itself has safeguarded the livestock population by its own voluntary disease control mechanisms, however, in view of the potential hazards that exist, the Committee on Infectious Diseases of Cattle, through a special subcommittee, recommends the following preliminary disease control mechanisms as they pertain to the use of artificial insemination of cattle.

The committee recommends to the United States Animal Health Association the following:

1. In recognition of the proportion of the cattle population of the USA (dairy 50% - beef 3%) artificially inseminated with frozen semen and the (a) number of animal contacts and (b) disease transmission potential implied thereby, the Committee intends to prepare a statement of minimum standards for bulls used in AI and for their semen which could be the basis for development by USDA of regulations for all bovine semen moving interstate.

2. The Committee intends to urge animal health officials of each state to require the registration of each processor of frozen semen and the maintenance by him of adequate “by bull” and “by collection” records.

3. Since it is so clearly evident that most frozen semen has the potential at some time for moving interstate, it is the intention of the Committee to develop a simple system of differential packaging so that semen limited to intra-herd use or to intra-state use in those states without AI regulations, can be readily recognized as semen not prepared for inter-state commerce.


The Committee recognizes that infectious disease research in cattle in this country is needed and that funds for such research have been reduced, and that such funds must be increased if the animal industry in this country is to meet future animal protein needs. Specific diseases which require immediate research efforts include:

1. Infectious keratitis (immunity and vector control studies)
2. Causes of abortion
3. Neonatal diseases

A continuing need exists for practical identification. The Committee again requests that the USAHA again recommend to the Secretary of Agriculture that he appoint a national task force to study intensively methods of animal identification particularly adaptable to cattle. This task force should include representation from all aspects of the cattle industry.

The Committee acknowledges the efforts of the National Preconditioning Coordinating Committee and endorses the concepts promoted by this organization.

In view of the problems encountered with bluetongue in cattle, the Committee requests that the USAHA recommend to the Secretary of Agriculture that funds be provided immediately for the construction of cattle isolation facilities at the
Animal Disease Laboratory in Denver.

Further this Committee proposes that the USAHA recommend to the Secretary of Agriculture that funding be provided on a long term basis for the operation of this laboratory.

The proposed off shore quarantine station was discussed and it was felt that legislation concerning this station had progressed beyond the point where committee action would be useful.

Please note supplement.

Respectfully submitted,

John B. Herrick,
Chairman

THE STATUS OF BOVINE AI IN THE U.S.A.
WITH SPECIAL REFERENCE TO ITS POTENTIAL
IN RESPECT TO DISEASE*

In 1969, in the United States, more than seven million dairy cattle and almost a million beef cattle are being bred by artificial insemination using semen that has, in most instances, been collected, processed, frozen, stored at locations of various distances from the point of use and then shipped to the point of use.

Preponderantly, the semen used during 1969 was collected and processed by about twenty-five organizations in the U.S.A. during the preceding year or so from bulls used exclusively for AI and kept at specialized farms. Some of the semen had been stored and frozen for several years—a few frozen ampules for as long as sixteen years. Some of the semen was custom collected between periods of natural service from bulls located on farms and ranches. Additionally, significant quantities of semen used originated from outside the U.S.A.

The DHIA Newsletter, May 1969, provides a detailed statistical study of those elements of the AI industry reporting statistics for 1968.1

Because of its several advantages, AI has gained gradual acceptance for breeding cattle, especially dairy cattle. Since 1950 while the dairy cattle population of the U.S.A. was decreasing from 24 million to 14 million, the gross production of milk increased 8% and then fell back to about the 1950 level. Production per cow during the period 1950 to 1968 increased from 5,314 to 9,000 lbs—an accomplishment for which AI deserves considerable credit through its contributions of education and management practices, superior genetics, fertility, and disease control. Insemination of beef cows has grown steadily since 1960.

The first fifteen years were characterized by a rapid increase in number of AI organizations. The second fifteen years have been characterized by an equally rapid decline in number of organizations with almost all becoming at least regional in scope and supplying some semen for national and international channels.

Bovine semen, by the very nature of its origin is seldom microbiologically

sterile for cells and entities other than spermatozoa. A remarkable number of pathogenic agents have the potential of making their way into bovine semen. Most of these pathogens can survive the processing, freezing, and storage conditions as readily as sperm cells.

To restrict collection of semen to pathogen-free bulls is a commendable ideal. To limit collection of semen to bulls established as specific pathogen free is a practical goal that has been the continuous objective of responsible elements in the AI industry. However, as time has passed and veterinary knowledge advanced, the list of specific pathogens of potential importance in AI grows longer and the complexities of pursuing the goal more complex.

One might have expected that in the advancing of AI the bulls used as semen sources would be maintained under conditions and regimens progressively more stringent and technically sophisticated. In fact, the typical level of surveillance of the bulls of the AI industry probably plateaued a decade ago, while the quite new practices of on-the-farm, and on-the-ranch custom collections of semen and one day or so a week visits of bulls at studs doing custom freezing of semen has become increasingly common. Some custom semen collectors are prepared to accept only responsibility for getting semen from a bull to a frozen ampule and for its storage and shipping. Consideration of the aspects of health of the bull are in the hands of the bull’s owner.

Standards and Regulations

At the federal level, USDA regulations exist establishing procedures and requirements for admitting semen into the U.S.A. from (1) Canada and Mexico and (2) countries where rinderpest or foot and mouth disease exist. It is interesting to note that these regulations specifically require consideration only of foot and mouth virus for semen entering from foot and mouth infected areas and from foot and mouth-free areas require only tests for brucellosis and tuberculosis. Tests or other consideration for diseases which are considered of most importance within the AI industry specifically: trichomoniasis, vibriosis, leptospirosis, and the brucellosis semen plasma test, are not required or mentioned in the federal regulations for entry of semen into the U.S.A. from foreign countries.

There are no federal regulations relating to AI within the U.S.A.

At the state level, four states, namely, Virginia, Mississippi, Montana, and Washington, regulate movement of bovine semen within their jurisdiction.

A recommended regulation and form for reporting was developed by a Sub-Committee on AI of the Committee on Infectious Diseases of Cattle of the U.S. Livestock Sanitary Association (1963). Although the regulations of these four states vary somewhat, all accept the USLSA Uniform Certificate for Intrastate or Interstate Shipment of Bovine Semen for Artificial Insemination.

Preponderantly, the AI industry in the U.S.A. has been guided and influenced by a voluntary code of minimum standards which a Special Committee on Animal Reproduction of the American Veterinary Medical Association recommended to and was adopted by the member organizations of the National Association of Animal Breeders in 1954.

No recent information exists relative to the degree of compliance with this code.
AI has the capability of being a powerful weapon in the control and elimination of certain infectious, venereal diseases of cattle and has the incidental value in the control of certain other systemic diseases because of obviating need for between herd movement of bulls for breeding purposes.

Preponderantly, AI has fulfilled its positive potential through implementation of routine and effective disease control measures at the bull level.

On the other edge, AI has the capability of disseminating certain infections more efficiently — faster, wider, and for longer — than is possible by any diseased bull in natural service.

Particularly in its early days before certain facts were recognized and certain procedures developed, AI was responsible for the spread of bivriosis and trichomoniasis in the U.S.A. The spread of brucellosis through AI was recorded in Denmark and England the spread of tuberculosis was recorded in France. This subject has been reviewed elsewhere.4

Whether the potentials of AI are a positive force or a negative force in livestock health is mostly a function of whether competent, technical procedures precede collection, processing, and freezing the semen from each bull.

REFERENCES


PREMUNIZATION WITH AN ATTENUATED
ANAPLASMA MARGINALE

K. L. Kuttler, DVM and H. Zaraza, DVM

Anaplasmosis immunization attempts, using killed antigens, have been shown to reduce the severity of subsequent infections, but generally incapable of preventing infection when exposure occurs\textsuperscript{1,2,4}. The absence of a complete, lasting, sterile immunity suggests that a premunizing approach might be indicated in enzootic areas where more complete protection is needed.

Theiler\textsuperscript{7}, as early as 1911, observed that infection with \textit{Anaplasma centrale} produced a degree of resistance to virulent anaplasmosis. Schmidt\textsuperscript{6}, in 1937, described a technique of premunition using virulent \textit{A. marginale}. He recognized age and ambient temperature as factors influencing the successful premunization of cattle. Over 1000 cattle were premunized with an estimated loss of only 1.3%. Kuttler\textsuperscript{3} reported the use of an \textit{A. marginale} isolate equally as mild as \textit{A. centrale}, which resulted in complete protection against virulent challenge, whereas \textit{A. centrale} merely reduced the severity of a virulent challenge.

Ristic \textit{et al}\textsuperscript{5} and Welter and Woods\textsuperscript{8} reported the development of an attenuated \textit{A. marginale} by serial passage of irradiated organisms in abnormal hosts. It was reported that virulence was lost for aged cattle and splenectomized calves.

This report deals with the attenuated \textit{A. marginale} and its use in Columbia, with the object of characterizing animal response to premunition as well as to evaluate its efficacy in preventing acute, severe anaplasmosis following challenge.

\textit{Materials and Methods}

Infection with the attenuated \textit{A. marginale} was induced in 21 Holstein-Friesian calves with an average age of 9.3 months, by the subcutaneous inoculation of 5 ml. of infected blood no more than 2 passages removed from the vaccine*. Twelve similar calves with an average age of 3.7 months were infected with virulent \textit{A. marginale}. Animal responses to these infections were compared (Table 1).

Infection with the attenuated \textit{A. marginale} was induced in 12 mature cattle of mixed breeding. Seven animals received 1 ml. of infected blood, and the remaining 5 were given 5 ml. All inoculations were made subcutaneously. Five mature cows were similarly infected by injecting 1 ml. of blood containing a virulent \textit{A. marginale}. Animal responses to these infections were compared (Table 2).

The comparative severity of animal response at 2 climatic zones to the attenuated \textit{A. marginale} was determined (Table 3). Fifteen Holstein-Friesian calves at Bogota, and 10 similar calves at Palmira, with a mean temperature of 14\degree C and 24\degree C respectively, were infected with the attenuated \textit{A. marginale} by the subcutaneous inoculation of 5 ml. infected blood not more than 2 passages removed from the vaccine.

Comparisons in Tables 1, 2 and 3 include low packed cell volume (PCV), high

\textsuperscript{*} Obtained from Diamond Laboratories. Des Moines, Iowa.
parasitemia and high complement-fixation (CF) titers for each group. An analysis of variance was conducted in each instance to determine the probability of error in mean differences.

The PCV response of vaccinated and non-vaccinated cattle to both artificial and natural challenge is presented in Figures 1, 2 and 3. Seven vaccinated and 5 non-vaccinated mature cattle were challenged with 1 ml. of a 0.75% suspension in 0.85% NaCl of washed red blood cells showing a 4% ascending \textit{A. marginale} parasitemia (Fig. 1). The challenging organism was isolated in Texas, and administered 50 days after the premunizing infection.

Two splenectomized calves, previously premunized with the attenuated \textit{A. marginale} were challenged with 20 ml. packed red blood cells showing a 0.2% parasitemia from a calf with virulent \textit{A. marginale} isolated in Texas. Two additional splenectomized calves, previously premunized with the attenuated \textit{A. marginale} were challenged with 5 ml. of blood showing a 0.2% parasitemia from a calf with a virulent \textit{A. marginale} isolated in Colombia (Figure 2). All 4 calves had been pre-munized about 4 months prior to challenge.

A total of 10 Holstein-Friesian calves, 5 vaccinated and 5 unvaccinated, were placed on known \textit{Anaplasma} infected premises and naturally exposed to anaplasmosis. The day on which the low PCV occured, if associated with other evidence of anaplasmosis, was designated Day 0. The PCVs were charted 42 days before and after Day 0 for evidence of significant fluctuation.

\textbf{Results}

Calves inoculated with the attenuated \textit{A. marginale} showed a significantly milder response when compared to reactions produced by the fully virulent organism (Table 1). Mature cattle infected with virulent \textit{A. marginale} developed an average low PCV of 12.4\% (Table 2). Mature cattle infected with the attenuated organism developed an average low PCV of 30.0\%. One death occurred among the 5 adults receiving the virulent organism. No deaths occurred among the 12 animals infected with the attenuated organism.

Of 15 calves inoculated at Bogota with the attenuated organism, only 7 reacted with diagnostic evidence of \textit{Anaplasma} infection (Table 3). The subsequent reactions observed in these 7 animals at Bogota were compared to similarly infected animals at Palmira. Significant differences in the severity of response failed to occur in these comparisons. There was a trend toward a milder response at Bogota, but the relative PCV (percent of normal) and parasitemia differences failed to reach significance. A significantly higher CF titer was recorded among animals quartered at Palmira.

The challenge results on 7 vaccinated and 5 non-vaccinated mature cattle inoculated with virulent \textit{A. marginale} of Texas origin is presented in Figure 1. The non-vaccinated cattle developed acute anaplasmosis with low PCV values and demonstrable parasitemias. One death occurred in this group. No apparent change in PCV values or other evidence of anaplasmosis was noted among the vaccinated animals.

The PCV responses and parasitemia of vaccinated, splenectomized calves, following exposure to two different \textit{A. marginale} field isolates, are presented in Figure 2. A Texas isolate did not produce a characteristic drop in PCV, although
considerable fluctuation occurred. The Colombian isolate produced a marked reduction in PCV at a time compatible with a challenge infection.

No appreciable differences could be detected between 5 vaccinated and 5 non-vaccinated calves receiving a field exposure to *A. marginale* in Colombia (Figure 3). The drop in PCV for both groups as illustrated in Figure 3 was also accompanied by an increase in the anaplasmosis CF titer.

**Discussion and Conclusions**

Infections produced by the attenuated *A. marginale* organism in calves and adult nonlactating cattle were less severe than infections resulting from virulent *A. marginale*.

Environmental factors are apparently involved in the course of premunizing infections with the attenuated *A. marginale*. This is best illustrated by a failure of known infective inoculums to produce a demonstrable *A. marginale* parasitemia in 8 of 15 calves, at Bogota. The remaining 7 calves developed evidence of infection which was slightly less severe although not significantly less than infections seen among 10 calves at Palamira.

Premunition or vaccinating infections in adult cattle with the attenuated *A. marginale* resulted in complete protection from a Texas isolate. Splenectomized and intact calves presumed to be carrying the attenuated organism reacted to challenge when exposed to a Colombian isolate by developing signs of acute anaplasmosis.

Mott observed that in some instances heavy challenge of carrier animals could result in a mild exacerbation of anaplasmosis signs. He and others have also noted that considerable variation in virulence exists among different *A. marginale* isolates. The results of these trials indicate that either the Colombian isolate is more virulent, resulting in a more pronounced secondary response following challenge, or that it represents an immunologically distinct strain. Differences between the Texas and Colombian isolates were not detected with the routine CF test, or by change in morphology of the *Anaplasma* body as seen with Giemsa stain.

**Summary:**

An attenuated *Anaplasma marginale* infection has been established in 21 calves and 12 mature cattle. The resulting infections were found to be significantly less severe than virulent *A. marginale* in 12 calves and 5 mature cattle. A slightly milder response to the attenuated *A. marginale* occurred in calves at Bogota with a mean temperature of 140°C when compared to calves similarly infected at Palmira with a mean temperature of 240°C.

Calves and mature cattle previously premunized with the attenuated organism appeared to be immune to virulent challenge using a Texas isolate of *A. marginale*. Experimental and natural challenge with a Colombian isolate resulted in evidence of acute anaplasmosis in both vaccinated and non-vaccinated animals.

**ACKNOWLEDGEMENTS**

This work was supported by grants from The Rockefeller Foundation and The U.S. Agency for International Development to The Institute of Tropical Veterinary Medicine, Texas A&M University. The Instituto Colombiano Agropecuario,
Laboratorio de Investigaciones Medicas Veterinarias, where the work was conducted contributed space, laboratories and use of equipment.

### TABLE 1
Comparative Response of Young Calves to Attenuated and Virulent *A. marginale*.

<table>
<thead>
<tr>
<th></th>
<th>No. of Animals</th>
<th>Avg. Age Months</th>
<th>Avg. Pre-Infection PCV %</th>
<th>Avg. Low PCV %</th>
<th>Avg. High Parasitemia %</th>
<th>High CF Titer</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated <em>A. marginale</em></td>
<td>21</td>
<td>9.3</td>
<td>29.5</td>
<td>21.3</td>
<td>1.3</td>
<td>1:525</td>
<td>0</td>
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<tr>
<td>Virulent <em>A. marginale</em></td>
<td>12</td>
<td>3.7</td>
<td>30.3</td>
<td>17.1</td>
<td>5.2</td>
<td>1:498</td>
<td>0</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>N.S.</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>N.S.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.S. = Not Significant

### TABLE 2
Comparative Response of Mature Cattle to Attenuated and Virulent *A. marginale*

<table>
<thead>
<tr>
<th></th>
<th>No. of Animals</th>
<th>Avg. Age Months</th>
<th>Avg. Pre-Infection PCV %</th>
<th>Avg. Low PCV %</th>
<th>Avg. High Parasitemia %</th>
<th>Avg. High CF</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated <em>A. marginale</em></td>
<td>12</td>
<td>37±22</td>
<td>37</td>
<td>30.0</td>
<td>0.9</td>
<td>1:640</td>
<td>0</td>
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<tr>
<td>Virulent <em>A. marginale</em></td>
<td>5</td>
<td>79±50</td>
<td>36</td>
<td>12.4</td>
<td>20.8</td>
<td>1:1930</td>
<td>1</td>
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<tr>
<td>Significance</td>
<td></td>
<td>N.S.</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.S. = Not Significant
### TABLE 3

Comparative Response of Intact Calves to Attenuated *A. marginale* at Bogota (14°C) and Palmira (24°C)

<table>
<thead>
<tr>
<th></th>
<th>No. of Animals Reacting</th>
<th>Avg. Pre-Infection PCV %</th>
<th>Avg. Low PCV %</th>
<th>Percent of Normal PCV</th>
<th>Avg. High Paras. %</th>
<th>Avg. High CF</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogota</td>
<td>15</td>
<td>36.9</td>
<td>29.3</td>
<td>79.4</td>
<td>0.69</td>
<td>1:290</td>
<td>0</td>
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<tr>
<td>Palmira</td>
<td>10</td>
<td>29.5</td>
<td>21.1</td>
<td>71.5</td>
<td>1.89</td>
<td>1:788</td>
<td>0</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

*N.S. - Not Significant*
FIGURE 1. PCV response of vaccinated and non-vaccinated cattle to needle challenge using a Texas *A. marginale* isolate.

* The day on which the low PCV occurred due to anaplasmosis (non-vaccinated).
FIGURE 2. PCV and parasitemia response of vaccinated splenectomized calves challenged with Texas and Colombian isolates.
FIGURE 3. PCV response of vaccinated and non-vaccinated calves to natural Anaplasma challenge in Colombia.
REFERENCES


ANAPLASTOSIS

Forrest E. Henderson
Veterinarian in Charge, Animal Health Division
Baton Rouge, Louisiana

Louisiana inaugurated the anaplasmosis study in January 1969. This was later than desired but, rather than postpone the study another year, we decided to go ahead. Basically, the study is based on the test and treat (TNT) approach.

Not only did we get off to a late start but the weather man seemed to be against us also. We only had one or two short-lived freezes prior to the starting date and, even worse for us — not for the cattlemen, of course — January and February, usually our coldest months of the year, recorded no freezing temperature at all. As a result, pasture grasses were evident in February, especially in South Louisiana, and mosquitos were active most of the year.

The abundance of grass during the days of medicated pasture supplement feeding caused the cattle not to be interested in consuming an adequate amount of the supplement. We had used salt, 650 pounds per ton, to prevent over consumption. This had to be reduced to one-half to assure adequate consumption and overcome the lure of lush grass pastures. All salt had to be eliminated in feed to one herd in South Louisiana that graze in marsh land where all water is brackish. In one other instance, the supplement was fed on a dry lot basis. Such an arrangement in Louisiana would not be practical for most cattlemen since it involves more labor and expense. This coming year, very little if any salt will be used in the pasture supplement. Salt will be added by the livestock owner as local conditions dictate.

Considering our late start and our trouble with vectors, the lure of lush pastures and too much seasoning in the feed, we feel we have made an impressive beginning. This successful beginning would not have been possible without the cooperation, assistance, consultation, and advice we have had from the USDA Animal Disease and Parasite Research Division, Fort Dodge, Pfizer, American Cyanimid, Louisiana State University Department of Veterinary Science, Cooperative Agricultural Extension Service, and other Department of Agriculture officials. Neither would this have been possible without the biologics furnished by Fort Dodge, and the medication furnished by Pfizer and American Cyanamid, and diagnostic equipment furnished by Henson, Wescott, and Dunning. Of course, we cannot overlook the splendid cooperation we have received from the livestock owners. We must not forget to mention the excellent work and assistance of the practicing veterinarians who have administered the anaplasmosis vaccine, at the expense of the owner, and who have kept us informed of the occurrence of anaplasmosis in the area where our field study is being conducted.

Dedication of certain professional people should be recognized now. Dr. F. B. Wheeler, State Veterinarian of Louisiana, has spent innumerable hours in planning, running, and on few occasions losing his temper in getting this project off a ground level.

Dr. Lon Foote of Louisiana State University has gone the extra step in going to the field to iron out certain technical problems in addition to his excellent technical work.
Dr. W. L. Trahan, Epidemiologist with the Animal Health Division, has picked up the pieces and worked out the mechanics of co-ordinating with all agencies, and, gentlemen, you know that is no small task.

Without the dedication of the three people mentioned this project would not have been accomplished in Louisiana.

Twenty-four (24) herds totaling 4,682 animals were tested; however, only 18 herd owners involving 4,079 head of cattle agreed to participate in the field study. These herds are located in 4 different areas of the State where anaplasmosis is known to be active. 1,874 reactors were treated and all 4,079 head of cattle were given 2 doses of Anapraz Vaccine. Reactors were vaccinated after treatment.

As of this date, we have not had a report of anaplasmosis being diagnosed in any of the 18 study herds. Practitioners and regulatory personnel report that in some areas of the State rather severe outbreaks of anaplasmosis have occurred this year. Some of these outbreaks have occurred in pastures adjoining those of our study herds.

We are presently in the process of surveying the herd owners to determine if they wish to continue the study, and to establish the date we plan to test their cattle and start the annual cycle on its second turn of testing and treating reactors, plus vaccination with Fort Dodge Anapraz Vaccine.

**LOUISIANA ANAPLASMOSIS FIELD STUDY**

**SUMMARY OF HERDS TESTED, TREATED AND VACCINATED**

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total herds on field study</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Total number of animals tested</td>
<td>4,079</td>
<td></td>
</tr>
<tr>
<td>No. of animals card test reactors</td>
<td>1,631</td>
<td>39.99%</td>
</tr>
<tr>
<td>No. of animals card test trace</td>
<td>114</td>
<td>2.79% Trace</td>
</tr>
<tr>
<td>No. of animals card test negative</td>
<td>2,334</td>
<td>57.22% Neg.</td>
</tr>
<tr>
<td>No. of animals C.F. test reactors</td>
<td>1,259</td>
<td>30.87%</td>
</tr>
<tr>
<td>No. of animals C.F. test suspect</td>
<td>207</td>
<td>5.07% Susp.</td>
</tr>
<tr>
<td>No. of animals C.F. test incomplete</td>
<td>20</td>
<td>9.49% Incomp.</td>
</tr>
<tr>
<td>No. of animals C.F. test negative</td>
<td>2,593</td>
<td>63.57% Neg.</td>
</tr>
<tr>
<td>(63 samples not C.F. Tested — Lost in shipment to Laboratory)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of animals treated</td>
<td>1,874</td>
<td>45.94% Treated</td>
</tr>
<tr>
<td>(Card test reactors, C.F. test reactors and suspects)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The card test proved to be highly effective for field use. In most cases the card test results were completed for the herd within 10 or 15 minutes after the last animal was bled. We are also encouraged about the accuracy of the card test, for limited sub-inoculation work has implied that as many, if not more, infected animals are being picked out by the card test as by the C.F. test.

If there are any questions, I will try to answer them. If I do not feel qualified to do so, I am sure that Dr. Tom Roby and Dr. Bill McCallon who worked with us are here.
CURRENT FIELD AND LABORATORY STUDIES
OF THE ANAPLASMOSIS CARD TEST

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B. R. McCallon**, D.V.M.
E. A. SchilP*, D.V.M.

The authors appreciate the cooperation and assistance of Dr. P. Becton, Federal Veterinarian in Charge, Arkansas; Dr. F. E. Henderson, Federal Veterinarian in Charge, Louisiana, and Dr. F. B. Wheeler, State Veterinarian, Louisiana. We also thank Dr. W. L. Trahan, Dr. W. H. Martin, their associates and Mr. J. C. Caudill for assistance in performing the card and complement fixation tests. The electron micrographs were furnished through the courtesy of Mr. R. L. Sealock, Beltsville Parasitological Laboratory.

In the opening address of the National Anaplasmosis Conference (1968) in Stillwater, Oklahoma, Dr. F. B. Wheeler, State Veterinarian, Louisiana, stated that the time to begin a “test and treat” program for the eradication of bovine anaplasmosis is NOW. At the 1968 National Anaplasmosis Conference, a report was given on the developmental investigation of a rapid card agglutination test as a serological diagnostic test for anaplasmosis. During the past year, a progress report was made to the U.S. Animal Health Association on our continuing investigations of the Anaplasmosis Card Test which employs an Anaplasma Buffered Antigen2,3. The results of these studies have been encouraging and, during the past year, a pilot study, which incorporated a “test and treat” program, was initiated in two states, Arkansas and Louisiana. It is our purpose at this time to present the laboratory and field (chute-side) results of this study, using the card agglutination test to identify cattle infected with Anaplasma marginale.

Since particle size and homogeneity often influence the serologic properties of agglutinating antigen, the morphologic characteristics of the Anaplasma Buffered Antigen also were considered. Thus, ultrastructure studies by electron microscopy were made to characterize the antigen and to formulate a standard antigen for comparison of future antigen preparations.

MATERIALS AND METHODS

Field evaluation trials:

Procedures for the complement fixation (CF) and card tests* were as previously described.1,2 Anaplasma-buffered antigen for these studies, prepared 3-6 months before use had been stored in sealed glass ampules at 6°C. Adequate antigen

* From the Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.
** From the Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, Federal Center Building, Hyattsville, Maryland 20782.
* The cooperation and supplies furnished through the courtesy of Dr. G. S. Warner and Mr. G. J. McMillion, Hynson, Westcott and Dunning, Inc., Baltimore, Md. 21201 are greatly appreciated.
for a day's testing was removed from the refrigerator and transferred to plastic
dispenser bottles. Ampules and dispenser bottles of unused antigen were returned
to the refrigerator each night. Antigen placed in the plastic dispenser bottles was
used for one week.

Tests were performed on blood samples from 715 cattle in a large herd in
Arkansas. This herd had a history of anaplasmosis and diagnosed cases had been
removed in previous years. Tests were also performed on blood samples from 4,605
cattle in 23 herds with histories of anaplasmosis, in Louisiana. Card tests of the
above samples were performed immediately at the farms; comparative CF tests were
performed at Beltsville, Md.

Laboratory studies:
At monthly intervals, the specificity and sensitivity of the card antigen were
tested using plasma from 60-70 cattle of known anaplasmosis status maintained at
the Beltsville Parasitological Laboratory.

The morphology of the anaplasma parasites after preparation and concen-
tration for card test antigen was examined and photographed using a Norelco EM
200 electron microscope*. Specimens for examination were prepared as follows.

Two-nil samples of sedimented antigen were fixed for 1 hour in 2% aqueous
osmium tetroxide at 0 C. They were immediately dehydrated for 15 minutes in 70
and 80% ethyl alcohol and for 30 minutes in 95% ethyl alcohol. Next, they were
placed for 30 minutes in 2 changes of absolute alcohol at room temperature
followed by 15 minutes in 3 changes of propylene oxide. Embedding was begun
with a 50-50 mixture of propylene oxide and epon 812 overnight. Final embedding
was in epon 812 and polymerization was accomplished by placing the specimen in
an oven at 60 C for 24 hours.6

Ultrathin sections of the samples were placed on grids and stained with 5%
uranyl acetate for 45 minutes. The staining was done under a 250-watt infra-red
heat lamp placed 18 inches above the samples. Final staining was in Reynold's lead
citrate for 5 minutes.7

RESULTS

Antigen specificity and sensitivity:
No decrease of antigenic specificity or reactivity was noted during the 6-month
observation period when the antigen was used in field testing or when it was used at
Beltsville to test blood samples from normal and anaplasmosis-infected cattle.
During these studies it was again noted as reported previously2 that the CF test
detected antibodies earlier than the card test, in the blood of recently anaplas-
mosis-infected cattle. Specifically, samples from three 5-year-old cows, inoculated
with 5 ml of anaplasmata-infected blood, were CF-positive 15 days after
inoculation. Card reactions, however, were positive on samples from one cow 15
days after inoculation and after 30 days in samples from the others. The interval
between positive CF reactions and card reactions appears to be even longer in

* Philips Electronic Instruments, Div. Philips Electronics and Pharmaceutical Industries, Inc.,
750 South Fulton Ave., Mount Vernon, N.Y.
recently infected calves. Blood samples from calves inoculated with 5 ml and 40 ml of anaplasmata-infected blood failed to react to the card test until 90 days postinoculation. The CF reactions, however, were positive in 15 days for the calf inoculated with 5 ml of infected blood and in 60 days postinoculation for the calf inoculated with 40 ml of infected blood.

Field evaluation trials:

Results of the card test indicate that the average anaplasmosis infection rate was 42.5% and 11.0% in the Louisiana and Arkansas herds, respectively. Table 1 lists the combined card and CF test results on all field samples. The data have been tabulated to show the relationship between CF titers and card test reactions on duplicate samples. Of 1,893 samples that were card-positive 418, or 22.1%, were CF-negative while the remaining 1,475, or 77.9% were reactive at various CF titer levels. It may also be noticed that 134 samples were classified as suspect to the card test. This classification was made on the basis of a minimal degree of visible agglutination. Since 91 or 67.9% of these samples were negative when tested by the CF method, the significance of these card test suspect reactions is under study and evaluation. There were 3,293 samples that were negative to the card test. Of these, 3,030 or 92% were also CF-negative.

TABLE 1

<table>
<thead>
<tr>
<th>CF Titer</th>
<th>Number Cattle Tested</th>
<th>Card Test Reactions</th>
<th>Percentage of Agreement*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Suspect</td>
</tr>
<tr>
<td>160</td>
<td>100</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>80</td>
<td>146</td>
<td>130</td>
<td>6</td>
</tr>
<tr>
<td>40</td>
<td>261</td>
<td>235</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>374</td>
<td>338</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>345</td>
<td>293</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>278</td>
<td>218</td>
<td>10</td>
</tr>
<tr>
<td>5&lt;</td>
<td>277</td>
<td>170</td>
<td>9</td>
</tr>
<tr>
<td>Neg.</td>
<td>3539</td>
<td>418</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>5320</td>
<td>1893</td>
<td>134</td>
</tr>
</tbody>
</table>

* Suspect card test reactions considered positive in percentage calculations

Electron microscope studies of antigen:

Electron micrographs of antigen samples are shown (Fig. 1-6). No remnants of erythrocytic stroma were observed in any of the preparations. At low magnification, the antigen appeared to be composed of numerous *A. marginale* substructures
(arrows, Fig. 1). Closer examinations at higher magnification showed that a number of distinct sizes and shapes of *A. marginale* were present. Some of the parasites were in much the same state as is usually seen in the host erythrocyte, that is, with the substructures (S) still intact, surrounded by a limiting membrane (LM, Fig. 2). Others appeared as individual anaplasma substructures with the pellicle, or outer membrane still intact as previously described\(^5\) (Fig. 3). Also in Fig. 3 the anaplasma substructure in the top right corner has apparently lost its pellicle and been partially ruptured, presumably by the antigen preparation process.

Small circular bodies (CB) approximately 100 μm in diameter were observed in many of the antigen electron micrographs. These were observed both inside and outside the anaplasma substructures. In Fig. 2, 3 circular bodies are visible inside the matrix of the anaplasmata but exterior to the substructures. In Fig. 3, a circular body may be seen within the lower substructure. In Fig. 4, 3 small elliptical bodies may be seen within a single substructure. In Fig. 5 there are several CB that appear attached to what may be a broken anaplasma substructure. Fig. 6 shows 2 anaplasmata in an erythrocyte. One body contains a single substructure, the other contains 4. Both are still within the host erythrocyte. The anaplasmata that contains 4 substructures also contains 2 CB. Since these CB were in the host erythrocyte before it was ruptured during antigen production, it would seem that they are indeed a part of the parasite and not artifacta.

**DISCUSSION**

In general, there are 3 ways the bovine anaplasmosis problem can be approached from an animal health viewpoint: (1) observe the disease and take no direct action other than treating sick cattle to help reduce economic losses, (2) apply the principle of premunition, which assumes that all susceptible cattle should be exposed to the disease in a mild form that will allow them to recover but remain carriers of *A. marginale* that might revert to virulence or (3) reduce the spread of anaplasmosis by identifying and treating infected cattle. As a supplemental precaution, herd owners could then elect to have all cattle negative to both the CF and card tests vaccinated with killed vaccine.\(^4\) One must realize however, that these vaccinated cattle will most probably be positive to both or either of the tests and remain so for a considerable length of time, especially if an annual "booster" inoculation is given. The third approach, a "test and treat" program, now seems more feasible than ever as data in this report show the card test to be a rapid and practical method of identifying infected cattle. Specifically, the card test correctly identified 85.2% of the anaplasmosis-infected cattle compared to the CF test results obtained in the laboratory. The comparison assumes the CF test to be 100% accurate.

Unfortunately no serological test for anaplasmosis or any other disease is 100% accurate in identifying infected cattle. The card and CF tests are no exceptions. This should not discourage us, however, for if we wait for 100% accuracy, we shall never begin to control and eradicate bovine anaplasmosis. We know that the card test has limitations in that card reactions appear later than CF reactions in the disease course. We also know that agglutinating antibodies, detectable by the card test, are slow in developing in young calves. However, isn’t it better to identify 85%
Fig. 1-5. Electron micrographs of sectioned anaplasmata found in card test antigen. Fig. 6. Electron micrograph of a sectioned bovine erythrocyte containing 2 anaplasmata. Circular bodies (CB), erythrocyte (E), limiting membrane (LM), and anaplasmata substructures (S). Magnification: Fig. 1. 14,000 x; Fig. 2. 60,000 x; Fig. 3, 4. 50,000 x; Fig. 5. 40,000 x; and Fig. 6. 36,800 x.
of our infected cattle than to wait for a perfect test and do nothing in the meantime? We believe the answer is obvious.

A critical evaluation of the accuracy of any test for anaplasmosis may only be achieved by subinoculating blood from reactor or nonreactor cattle into susceptible cattle. Results of inoculations of blood from cattle that were CF-positive and suspect but card-negative as well as CF-positive and card-positive have been reported in a previous study. More such inoculations are certainly needed. During the present study, a limited number of subinoculations were made from cattle that were CF-positive, card-negative and CF-negative, card-positive. Of 4 CF-positive, card-negative cattle, 3 were proved to be infected by subinoculation; but 6 of 8 cattle, which were CF-negative, card-positive, were also infected. A possible explanation may be that the CF test is superior in detecting early anaplasmosis infection while the card test is more accurate in disclosing long term infections.

Aside from its usefulness in testing, the unstained card test antigen has been most useful for ultrastructure studies of the parasite. Literally thousands of parasites are present in Anaplasma Buffered Antigen prepared for study with the electron microscope, while only a few intraerythrocytic parasites can be observed in each electron microscope field when intact erythrocytes are used as a source material. The large numbers of parasites present in the card test antigen, make it possible not only to observe many more parasites per field, but also to compare them directly, searching for clues as to their mode of development. On the basis of these studies, a developmental stage, the CB stage, has been described.

SUMMARY

The rapid card agglutination test for bovine anaplasmosis is proving to be an accurate and practical method for use in a test and treat program. Plasma and serum samples from 5,320 cattle in Arkansas and Louisiana were tested by both the anaplasmosis card agglutination and complement-fixation (CF) tests for comparative studies. Eighty-five percent of the samples that were CF-positive or suspect reacted to the card test antigen. Ninety-two percent of the samples that were CF-negative were also card test negative. The card agglutination test made it possible to separate 85% of the CF reactive cattle from the negative cattle on the day the herd was tested.

Examination of the card test antigen by electron microscopy has shown it to be composed of *Anaplasma marginale* parasites free of erythrocytic stroma. The heavy concentration of parasites in the antigen facilitates studies of their morphologic characteristics. A small circular body approximately 100 µm in diameter, possibly an early developmental stage not previously described, was observed in the antigen.

REFERENCES


LABORATORY AND FIELD TRIALS
WITH AN
ATTENUATED ANAPLASMA MARGINALE VACCINE

C. J. Welter*; Ph.D.
Miodrag Ristic**, D.V.M., Ph.D.

The development of an attenuated live Anaplasma marginale vaccine based upon selection of an avirulent variant by serial passage of irradiated organisms in nonbovine ruminants has been reported.1 The efficacy and safety of the vaccine has been demonstrated in field and laboratory tests comprising more than 5,000 cattle.1,2

The purpose of the present study was to: 1) further determine immunoserologic and immunogenic responses of cattle to the vaccine when it is used singly or in combination with a killed vaccine, 2) determine duration of immunity conferred by the vaccine, 3) ascertain in vivo stability of the vaccine, and 4) investigate sensitivity of the vaccine strain to tetracycline.

MATERIALS AND METHODS

Herefords 2 and 4 years old were used for immunoserologic and immunogenic studies. An attenuated1 and a killed3 vaccine were administered singly or in combination to these cattle. Animals used in other experiments were of Holstein-Freisen or mixed breeds and ranged in age from 3 months to 7 years. A virulent strain of A. marginale originally secured from Florida1 was used to challenge immunity of vaccinated animals. A capillary tube agglutination (CA) test was used to detect and titrate anti-anaplasma antibodies4,5.

RESULTS

Immunoserologic studies on vaccinated and non-vaccinated cattle and responses of these animals to challenge with virulent A. marginale are shown in figure 1 (A, B, C, and D). In susceptible animals (Fig. 1-A) which were inoculated with attenuated vaccine, serologic response was noted approximately 6 weeks after vaccination, rose to a maximum titer (1:16) 2 months thereafter, and then rapidly subsided, with reactions occurring only in undiluted serum at approximately 6½ months after vaccination. When the three animals of this group were each challenged by an intramuscular injection of 1.0 ml of virulent blood containing approximately 25% infected erythrocytes, no serologic, hematologic and clinical abnormalities were noted in any of the animals. Two Herefords in group B served as controls for these animals. Based upon their history and lack of reactivity in the CA test, animals of group B were susceptible to A. marginale. When the challenge, identical to that used on animals of group A, was given to the 2 animals of group B,
both developed severe anaplasmosis and one died approximately 6 weeks after the challenge. The infection in these 2 animals was followed by a rapid rise of the CA antibody titer. Cattle of group C received two injections of killed anaplasma vaccine on days 1 and 45, respectively. The animals developed a transitory response in the CA test which was detectable between 15 and 70 days following vaccination. On challenge, using 1.0 ml of 5% infected erythrocytes, five months after administration of the first vaccine dose, the animals developed severe anaplasmosis and two of them died approximately 30 days following the challenge. The remaining animal made gradual recovery from the disease during the four month’s period after challenge.

The animals of group D received both vaccines. Two doses of the killed vaccine were administered on days 1 and 45, respectively. A single dose of the attenuated vaccine was given approximately 2 weeks after the second dose of the killed vaccine. The pattern of serologic response of these animals was similar to that demonstrated by animals of group A which received the attenuated vaccine only. On challenge with 1.0 ml of 12% infected erythrocytes, the animals of this group were found completely protected similar to those of group A. No hematologic and clinical abnormalities were noted in any of the animals.

The study of stability of the vaccine strain in an adult animal in which it was maintained during a 6 to 36 month period is described in table 1. Regardless of blood volumes which ranged from 1.0 ml to 150 ml subinoculated from vaccinated to susceptible animals, none of the latter animals developed clinical signs of anaplasmosis. These subinoculations were made by intravenous, intramuscular and subcutaneous routes.

Duration of immunity to anaplasmosis conferred by the attenuated vaccine and measured by the presence of a reaction in the CA test, is described in table 2. In animals which were vaccinated at 1 month to 1 year of age, the protection to anaplasmosis determined 3 to 6 months later was 100%; in mature cattle vaccinated at 3 to 5 years of age the protection determined 3 to 36 months later varied between 82% and 100%. One vaccinate when challenged with 5 ml of virulent blood from an A. marginale carrier 36 months after vaccination was completely resistant.

The effect of parenteral treatment with tetracycline upon elimination of the attenuated and virulent strain of anaplasma from cattle is shown in table 3. Daily administration of 8.5 mg. tetracycline per pound of body weight to vaccinated cattle for 4 days completely destroyed the attenuated strain. Daily administration of 10 mg. tetracycline per pound of body weight for 7 days completely destroyed the attenuated as well as the virulent strain.

The results of a large field vaccination study on cattle which were imported from the United States into Venezuela are described in table 5. This experiment involved 2700 beef cattle of mixed breeds. The animals were vaccinated enroute to Venezuela. After arrival at their destination in a tropical area of Venezuela, the vaccinated cattle were positive for the CA test. The losses in this herd were reduced from approximately 9.5% in 1967, when non-vaccinated cattle were imported, to 2.5% when vaccinated cattle were imported.
Certain aspects of immunity and protection in anaplasmosis have been tested and the results revealed in Figure 1 (A-D). There are 3 interesting features which highlight the results of these experiments: 1) the protection conferred by the attenuated vaccine was solid, 2) the killed vaccine did not induce a significant degree of protection against the challenge of 1 ml which may have been too severe and 3) application of the killed vaccine did not in any way interfere with subsequent use of the attenuated vaccine. Of particular interest is the fact that serum antibodies, stimulated by the killed vaccine, in no way interfered with the establishment, growth, and multiplication of the vaccine strain of anaplasma. The virulent strain has also been reported to grow and establish itself in cattle immunized with killed vaccine.3

It is within the frame of possibility that in animals which were vaccinated for longer periods of time the balance between the host's defense forces and the parasite can eventually be upset and altered. Under various circumstances, humoral (serum antibody) and cellular defenses could induce gradual mutation of the vaccine strain and subsequent selection of a variant type. The latter organism may assume pathogenic and other organismal properties which would be slightly different from those of its prototype. The results of experiments summarized in table 1 indicated that the vaccine strain remained apathogenic in a vaccinated animal for as long as 36 months, the longest test period studied. For all practical purposes and in the absence of reinfection with a virulent anaplasma strain, protection resulting from a single dose of the vaccine may be expected to last for at least 3 years.

A relatively high degree of sensitivity of the vaccine strain to destruction by tetracycline may serve a useful purpose and have a practical application under certain field conditions. More specifically, it would mean that in a given situation, when it becomes desirable that a vaccinated animal be moved from an endemic area into a disease-free area, such an animal may be freed from the vaccine strain within a week's period. Studies by several workers have shown that an animal becomes serologically negative (CA test) shortly after the anaplasma was destroyed by treatment.6,7

The results of the field vaccination experiment, which involved 2,700 cattle, demonstrated several interesting qualities of the vaccine. The animals were given the vaccine while on the ship enroute to Venezuela. Under the circumstances, it could be expected that the animals have been subjected to various stress effects associated with the transport. These stresses, however, did not have any demonstrable adverse effect on the process of immunization. Shortly after arrival in Venezuela the animals were exposed to tropical climate and blood sucking arthropods known to be carriers of anaplasma and other blood parasites. In spite of this unfavorable environment, no side effects, spontaneous reversion to virulence, or any other adverse effects were noted. The overall mortality rate was greatly reduced in vaccinated animals in comparison to the non-vaccinated animals imported during the previous years.
ANAPLASMA MARGINALE VACCINE

FIGURE I

IMMUNO-SEROLOGIC RESPONSES OF VARIOUS GROUPS OF CATTLE TO CHALLENGE WITH VIRULENT A. MARGINALE.

Challenge - 1.0 ml Virulent Blood.
# TABLE I

## IN VIVO STABILITY OF ATTENUATED ANAPLASMA MARGINALE

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Months Interval *</th>
<th>M.L.</th>
<th>Route</th>
<th>Packed cell V. Initial</th>
<th>Packed cell V. Low</th>
<th>Peak Parasitemia</th>
<th>Symptoms</th>
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<tr>
<td>2610</td>
<td>6</td>
<td>150</td>
<td>I.V.</td>
<td>37</td>
<td>10</td>
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<tr>
<td>279</td>
<td>8</td>
<td>5</td>
<td>S.C.</td>
<td>38</td>
<td>12</td>
<td>45</td>
<td>No</td>
</tr>
<tr>
<td>280</td>
<td>8</td>
<td>5</td>
<td>I.M.</td>
<td>40</td>
<td>23</td>
<td>12</td>
<td>Signs</td>
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<tr>
<td>281</td>
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<td>S.C.</td>
<td>38</td>
<td>20</td>
<td>26</td>
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<tr>
<td>691</td>
<td>18</td>
<td>10</td>
<td>S.C.</td>
<td>30</td>
<td>12</td>
<td>38</td>
<td>Anaplasmosis</td>
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<tr>
<td>442</td>
<td>28</td>
<td>5</td>
<td>S.C.</td>
<td>36</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>204</td>
<td>36</td>
<td>1</td>
<td>S.C.</td>
<td>36</td>
<td>32</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>36</strong></td>
<td><strong>18</strong></td>
<td><strong>22</strong></td>
<td></td>
</tr>
</tbody>
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* Number months interval between vaccination of donor cow and subpassage to other cows.
<table>
<thead>
<tr>
<th>Age Group</th>
<th>Time of Vaccination</th>
<th>Number of Cattle</th>
<th>Test Duration (Months)</th>
<th>Percent Serologically Positive</th>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>25</td>
<td>82</td>
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<td>5 Years</td>
<td></td>
<td>3</td>
<td>20</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>3 Years</td>
<td></td>
<td>20</td>
<td>16</td>
<td>95</td>
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<td>9</td>
<td>3</td>
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<td>6</td>
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<td></td>
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<td>5</td>
<td>4</td>
<td>100</td>
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<td>3-5 Months</td>
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<td>4</td>
<td>100</td>
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<tr>
<td>1 Month</td>
<td></td>
<td>2</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Anaplasma</td>
<td>Group</td>
<td>Total Cattle</td>
<td>Tetracycline Treatment</td>
<td>No. Serologically Positive Cattle Ten Weeks Post-Treatment</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>--------------</td>
<td>------------------------------</td>
<td>------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>$2\frac{1}{2}$ mg./lb. daily - 3 days</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>5 mg./lb. daily - 3 days</td>
<td>3</td>
</tr>
<tr>
<td><strong>Attenuated</strong></td>
<td>3</td>
<td>6</td>
<td>$8\frac{1}{2}$ mg./lb. every other day - 3 days</td>
<td>2</td>
</tr>
<tr>
<td><strong>(Vaccinated)</strong></td>
<td>4</td>
<td>2</td>
<td>$8\frac{1}{2}$ mg./lb. daily - 4 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>10 mg./lb. daily - 3 days</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>10 mg./lb. daily - 7 days</td>
<td>0</td>
</tr>
<tr>
<td><strong>Virulent</strong></td>
<td>7</td>
<td>3</td>
<td>5 mg./lb. daily - 3 days</td>
<td>3</td>
</tr>
<tr>
<td><strong>(Carriers)</strong></td>
<td>8</td>
<td>5</td>
<td>10 mg./lb. daily - 3 days</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7</td>
<td>10 mg./lb. daily - 7 days</td>
<td>0</td>
</tr>
</tbody>
</table>
### TABLE 4

**VACCINATION OF CATTLE ON ROUTE FROM THE U.S. TO VENEZUELA**

<table>
<thead>
<tr>
<th>Breed &amp; Age</th>
<th>No. Vaccinated</th>
<th>No. Control</th>
<th>Percent Cattle Positive in the Ca Test</th>
<th>Mortality Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Vaccination</td>
<td>After Vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef 2-6 Yrs.</td>
<td>2400</td>
<td>300</td>
<td>1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

* No Vaccination
SUMMARY

Immunoserologic and immunogenic studies with an attenuated anaplasma vaccine demonstrated that it conferred solid protection to challenge with the virulent *A. marginale*. The use of a killed vaccine prior to application of the attenuated vaccine did not reduce the efficacy of the attenuated vaccine. Both susceptible control animals and those inoculated only with a killed vaccine succumbed on challenge to clinical anaplasmosis. No indication of a reversion to virulence of the vaccine strain was noted in cattle in which the strain was maintained for as long as 36 months. As judged by the persistence of the CA reaction in vaccinated animals, the protection conferred by a single dose of the attenuated vaccine is expected to last for at least 3 years.

The vaccine strain can be destroyed by parenteral administration to vaccinated animals of 4 daily doses of 8.5 mg/lb of tetracycline. The safety and efficacy of the vaccine was demonstrated in a large field vaccination experiment involving 2,700 cattle.

REFERENCES


SERIAL PASSAGE OF AN ATTENUATED
ANAPLASMA MARGINALE
IN SPLENECTOMIZED CALVES

K. L. Kuttler, DVM

The description of an attenuated Anaplasma marginale by Ristic et al. and Welter and Woods, suggests that an effective, safe premunization program for the control of bovine anaplasmosis might now be possible. Since a parasitemia does occur following the inoculation of this organism, the possibility of cow to cow transfer by natural vectors cannot be entirely eliminated. For this reason 12 serial passages have been made in splenectomized calves to determine if any evidence of altered virulence could be detected by this procedure.

Materials and Methods

Attenuated A. marginale infections were serially induced in each of 11 splenectomized, male, Holstein-Friesian calves averaging 202 ± 49 days of age. The first passage was made from vaccine obtained from the producing laboratory. This vaccine had been stored several months at -40°C before use. A total of 10 ml. of this material was inoculated intravenously (I.V.) and 10 ml. subcutaneously (SC). A second passage was made from the infected blood of the first passage calf. This process of serially inoculating calves was continued until an eleventh passage was completed. The size, route of inoculation, and parasitemias of each inoculum is presented in Table 1.

A second phase of this study consisted of comparing the relative severity produced by twelfth and second passage attenuated Anaplasma. Group 1 consisted of 4 splenectomized, male, Holstein calves with an average age of 94 ± 5 days. They were inoculated with infected blood showing a 1% parasitemia from an ascending infection occurring in the eleventh passage calf (2468). The inoculum consisted of 5 ml. of a 30% washed red blood cell suspension in 0.85% NaCl, injected SC.

Group 2 consisted of 4 splenectomized, male, Holstein calves with an average age of 102 ± 4 days. They were inoculated with infected bovine blood showing a 0.5% parasitemia from an ascending infection occurring in a first calf passage. The inoculum consisted of 5 ml. of a 60% washed RBC suspension in 0.85% NaCl injected SC. This first passage calf had been infected with material obtained from Mexico City and held frozen for 5 months at -40°C.

Each of the 8 calves used in this trial were given 5 ml. Spirotripan Fuerte I.V. at the time infected red cells were injected, to minimize the possibility of Eperythrozoon infection which might alter the course and severity of the induced A. marginale infection.
In the first phase of 11 serial passages blood samples were collected twice weekly to monitor the course of infection. During the second phase, blood samples were collected three times a week until a parasitemia occurred and then daily until recovery or death.

Complement-fixation (CF) tests were conducted weekly using the basic procedures outlined by the USDA\(^1\), but employing a microtechnique for titrations\(^3\). Packed cell volume determinations were made on all samples, using a micro-hematocrit centrifuge. Giemsa stained blood smears were used to determine parasitemias.

An analysis of variance was used for the determination of statistical differences. Averages and statistical evaluation of complement-fixation serum titers were made on logarithmic transformations of the dilution factors.

**Results**

The results of 11 serial passages are tabulated in Table 1. The incubation time is recorded as days required for the development of diagnostic evidence of anaplasmosis. The first passage, using vaccine material which had been frozen several months, produced infection after a prolonged incubation time of 47 days. Thereafter the incubation was much shorter. No significant correlation between passage level and high parasitemias or low PCV was evident. The seventh passage calf died of acute anaplasmosis, but the ninth passage calf developed a relatively mild reaction with a low PCV of only 17%.

Comparisons between groups 1 and 2 of phase 2 are presented in Table 2. The average incubation time was almost identical between the 2 groups. No significant difference in pre-infection PCV was noted. The average parasitemia of 36.8% observed in group 1 was significantly greater than the average of 3.6% observed in group 2. The average low PCV of 9.5% observed in group 1 was significantly lower than the 19.5% observed in group 2. There was no significant difference in CF titers. One animal in group 1 died of acute anaplasmosis. No deaths occurred in group 2.

**Discussion and Conclusions**

A second rather than a first passage was compared to the twelfth in order to standardize the inoculum used to induce infection. The vaccine material had been frozen, the parasitemia was unknown, and was from a non-bovine host. It would have been difficult and probably impossible to have standardized the vaccine as obtained with the twelfth passage material, to give a comparable inoculum.

Even though no clear pattern of reversion could be detected in the 11 serial passages, a comparison of reactions produced by twelfth and second passage material in 2 groups of 4 animals each clearly shows a difference. A reversion to virulence is indicated by the increase in parasitemia and the lower PCV observed in calves at the twelfth passage level.

This experiment does not prove that such a reversion would occur following 12 serial passages in intact calves under natural conditions. It does indicate a potential for reversion.

The number of susceptible cattle in contact with vaccinated animals, their age, and possibly breed, the type and number of vectors present, and general
management practices are all factors which might influence the serial passage of this attenuated organism in nature, and the rate of reversion to virulence.

In enzootic areas where premunization is the method of choice, the return to virulence is mostly of academic interest. When faced with the decision of whether to depend on premunition with a virulent organism by natural means on a sporadic basis or to vaccinate all calves with an attenuated organism, the choice is clearly in favor of the latter.

A potential for reversion does exist, and in areas of low anaplasmosis incidence, the introduction of even an attenuated agent might be hazardous when suitable vectors are present to produce serial transmission. The indiscriminate use of such a vaccine in some areas could establish infection where it previously had not existed.

Summary

Twelve serial passages of an attenuated Anaplasma marginale were made in splenectomized calves by blood inoculation. The severity of infection produced at the twelfth passage level in 4 splenectomized calves was compared to the infection occurring in 4 similar calves at a second passage level. Significantly higher parasitemias and lower packed cell volumes occurred in the twelfth passage group, suggesting an increased virulence. No deaths occurred among animals of the second passage group whereas 1 of 4 died in the twelfth passage group.

ACKNOWLEDGEMENTS

This work was supported by grants from The Rockefeller Foundation and The U.S. Agency for International Development to The Institute of Tropical Veterinary Medicine, Texas A&M University. The Instituto Colombiano Agropecuario, Laboratorio de Investigaciones Medicas Veterinarias, where the work was conducted contributed space, laboratores and use of equipment.

REFERENCES


<table>
<thead>
<tr>
<th>Passage Level</th>
<th>Animal No.</th>
<th>Size, Date, and Route of Inoculum</th>
<th>Parasitemia Of Inoculum %</th>
<th>Incubation (Days)</th>
<th>Pre-Infect. Normal PCV %</th>
<th>High Paras.%</th>
<th>High CF</th>
<th>Low PCV %</th>
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<tbody>
<tr>
<td>1</td>
<td>093</td>
<td>10 ml. I.V., 10 ml. S.C. Lot 8E Diamond Vaccine (7/5/67)</td>
<td>47</td>
<td>35</td>
<td>4</td>
<td>1:2560</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>2 ml. S.C. blood from 093 (8/25/67)</td>
<td>3</td>
<td>17</td>
<td>30</td>
<td>7</td>
<td>1:1280</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>511</td>
<td>10 ml. I.V. blood from 18 (2/14/68)</td>
<td>1</td>
<td>12</td>
<td>40</td>
<td>15</td>
<td>1:640</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>513</td>
<td>10 ml. I.V. blood from 511 (3/8/68)</td>
<td>8</td>
<td>10</td>
<td>44</td>
<td>50</td>
<td>1:1280</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>514</td>
<td>10 ml. I.V. blood from 513 (3/22/68)</td>
<td>26</td>
<td>10</td>
<td>33</td>
<td>10</td>
<td>1:640</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>518</td>
<td>10 ml. I.V. blood from 514 (4/8/68)</td>
<td>8</td>
<td>14</td>
<td>34</td>
<td>5</td>
<td>1:320</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>522</td>
<td>10 ml. I.V. blood from 518 (4/27/68)</td>
<td>2</td>
<td>16</td>
<td>30</td>
<td>30</td>
<td>1:320</td>
<td>9</td>
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<tr>
<td>8</td>
<td>34</td>
<td>10 ml. I.V. blood from 522 (5/17/68)</td>
<td>12</td>
<td>10</td>
<td>34</td>
<td>30</td>
<td>1:2560</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>10 ml. I.V. blood from 34 (5/31/68)</td>
<td>22</td>
<td>11</td>
<td>38</td>
<td>2</td>
<td>1:640</td>
<td>17</td>
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<tr>
<td>10</td>
<td>30</td>
<td>10 ml. I.V. blood from 35 (6/14/68)</td>
<td>0.5</td>
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<td>39</td>
<td>32</td>
<td>1:1280</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>2468</td>
<td>10 ml. I.V. blood from 30 (9/14/68)</td>
<td>1.0</td>
<td>23</td>
<td>30</td>
<td>10</td>
<td>1:1280</td>
<td>13</td>
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</table>
### Table II
Comparative Reaction of 12th and 2nd Passage Attenuated *Anaplasma*

<table>
<thead>
<tr>
<th>Passage Level</th>
<th>Animal No.</th>
<th>Parasitemia %</th>
<th>Incubation (Days)</th>
<th>Pre-Infect.</th>
<th>High</th>
<th>High CF</th>
<th>Low</th>
<th>PCV %</th>
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<tr>
<td>12</td>
<td>2568</td>
<td>1.0</td>
<td>13</td>
<td>31</td>
<td>50</td>
<td>1:1280</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3368</td>
<td>1.0</td>
<td>20</td>
<td>36</td>
<td>28</td>
<td>1:1280</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3568</td>
<td>1.0</td>
<td>15</td>
<td>44</td>
<td>65</td>
<td>1:80</td>
<td>9 died</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3668</td>
<td>1.0</td>
<td>15</td>
<td>38</td>
<td>4</td>
<td>1:1280</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Averages</td>
<td></td>
<td></td>
<td>15.75</td>
<td>37.25</td>
<td>36.8</td>
<td>1:640</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3068</td>
<td>0.5</td>
<td>14</td>
<td>37</td>
<td>7.0</td>
<td>1:1280</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3168</td>
<td>0.5</td>
<td>20</td>
<td>38</td>
<td>3.0</td>
<td>1:640</td>
<td>17</td>
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<tr>
<td>2</td>
<td>3268</td>
<td>0.5</td>
<td>14</td>
<td>36</td>
<td>1.2</td>
<td>1:1280</td>
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<tr>
<td>2</td>
<td>3868</td>
<td>0.5</td>
<td>14</td>
<td>45</td>
<td>3.0</td>
<td>1:640</td>
<td>20</td>
<td></td>
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<tr>
<td>Averages</td>
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<td></td>
<td>15.5</td>
<td>39.0</td>
<td>3.6</td>
<td>1:905</td>
<td>19.5</td>
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<td></td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.05</td>
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<td>P&lt;0.01</td>
<td></td>
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</table>

**Inoculum:**

Calves 2568, 3368, 3568 and 3668 at the 12th passage level received 5 ml. subcutaneously washed erythrocytes as a 30% suspension in 0.85% NaCl. These RBCs showed a 1% parasitemia from an ascending *Anaplasma* infection in animal 2468.

Calves 3068, 3168, 3268 and 3868 at the 2nd passage level received an equivalent inoculum consisting of 5 ml. S.C. washed erythrocytes as a 60% suspension in 0.85% NaCl. These RBCs showed a 0.5% parasitemia from an ascending *Anaplasma* infection. 5 ml. Spirotripan-Fuerte was injected I.V. in each of the 8 calves at the time they received the infecting inoculum.
PREVALENCE OF ANAPLASMA MARGINALE INFECTION IN CALIFORNIA DEER AS MEASURED BY CALF INOCULATION AND SEROLOGIC TECHNIQUES


Any plans or programs for the control or eradication of anaplasmosis in the continental United States would be influenced or even nullified by the existence of a persistent reservoir of Anaplasma marginale infection in non-bovine species such as deer. This would be particularly true if it can be shown that a cycle of infection between deer and ticks can occur in the absence of cattle.

The white-tailed deer that are native to the Southern and Eastern sections of the United States have been experimentally infected with A. marginale of bovine origin. In studies with one type of white-tailed deer (Dama virginiarum), it was shown that the Anaplasma parasite could invade mature deer erythrocytes that had been transfused into splenectomized calves with acute anaplasmosis.1 Inoculation of mature intact (Dama virginiarum) deer with bovine A. marginale produced clinically mild infections in which as many as 59% of the deer erythrocytes contained marginal bodies, but the length of the carrier state was not determined.2

Both acute and mild infections resulted when splenectomized Odocoileus virginianus deer were inoculated with A. marginale.3,4,5 Serial passage of the parasite in this deer produced some infections in which 95% of the erythrocytes contained marginal bodies.6

Information concerning the prevalence of natural infection in white-tailed deer is meager. One report states that Anaplasma infection was not found in 262 white-tailed deer from 9 southeastern states when blood samples were inoculated into splenectomized calves.7

There is ample evidence, however, that a well-adapted host-parasite relationship exists between A. marginale and the black-tailed deer (Odocoileus hemionus colombianus) of the Coast Range area of California. A. marginale can be transferred readily by blood inoculation from deer to calf, calf to calf, calf to deer, and from deer to deer.8 A. marginale from naturally infected carrier cattle will readily induce infection in deer,9 and the carrier state was demonstrated 11 months after inoculation.10 Anaplasmosis has also been produced in calves with ticks obtained from black-tailed deer.11

Studies of the prevalence of natural Anaplasma infection in California deer are summarized in Table 1. A compilation of the studies of these various investigators showed that blood from 94 deer inoculated singularly or as pooled samples into 43 test cattle resulted in a 72% transmission rate.12,13,8,14,15 Thus, there is evidence that anaplasmosis is a well-established latent infection in California black-tailed deer

* From the School of Veterinary Medicine, University of California, Davis, Calif. 95616.
** From the Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.
because the sampling referred to occurred over a 30-year period and at 4 different geographical sites. These previous studies, however, may not be an accurate representation of the extent of latent Anaplasma infection in California deer because the age of the deer in many cases was not determined, blood from more than one deer was often pooled before calf inoculation, and in most instances deer blood was held one to 10 days before calf inoculation.

The limited number of deer represented in Anaplasma prevalence studies is a reflection of the costly and time consuming calf inoculation procedure used to detect carriers. The complement fixation test is recognized as an accurate tool for the detection of Anaplasma carriers among cattle but the results of tests with deer sera have been shown to be misleading and unreliable. The capillary tube agglutination test was reported to be 97% accurate when applied to Anaplasma negative sera of big game animals but there are no reports of its use on sera from naturally infected deer. If an accurate serologic test was available for the testing of deer, the significance of deer as a source of Anaplasma infection could be more readily determined.

The present study was conducted to provide additional information on the prevalence of Anaplasma infection in California black-tailed deer. In order to obtain more meaningful results than those of past deer surveys, individual deer blood samples were inoculated into individual test calves within an hour after collection. In this study there was an opportunity to compare the accuracy of the complement fixation, capillary tube agglutination and card agglutination tests on sera of infected and non-infected deer. The 3 serologic tests were also compared for accuracy on sera from calves infected with Anaplasma of deer origin.

MATERIALS AND METHODS

Study areas. Two study areas approximately 5 miles apart were used for sources of deer blood samples. The University of California Field Station at Hopland in Mendocino County was one area. This station consists of nearly 5,000 acres of hill and mountain grazing land, which has not been available to cattle since 1952 except for 2 or 3 occasions when small groups of cattle were pastured for 3-month periods. Sheep are raised on this range land, which also has a large population of Columbian black-tailed deer (Odocoileus hemionus columbianus), the common species on California’s coastal mountain area. Thirty-three deer were collected on the Hopland Station during the period of October 1968 to May 1969.

The second study area, also in Mendocino County, was a privately owned cattle ranch where several clinical cases of anaplasmosis occur each year. Eight deer were collected on this ranch in May 1969.

Collection procedure. Four deer were trapped alive and blood was obtained by jugular venipuncture. The other 37 deer were shot in the head or neck and blood was drawn immediately by cardiac puncture. A portion of the blood from each deer was placed in tubes and permitted to clot for serum separation while the remainder was mixed with heparin to prevent clotting.

Experimental animals. Holstein steer calves 2 to 3 months of age were obtained from a single source. The calves were not splenectomized and were negative to each of the 3 serologic test methods prior to inoculation with deer blood.

Each calf was inoculated subcutaneously with 15 to 35 ml of blood from a
single deer. The time interval between collection of the blood from the deer and inoculation of calves seldom exceeded one hour.

The calves were maintained in fly-proof enclosures following inoculation. Beginning 14 days after inoculation, blood was obtained from calves 3 times per week for Anaplasma body detection and once per week for serologic studies. All calves were maintained until transmission of anaplasmosis was demonstrated or until animals had gone at least 90 days past the date of inoculation without evidence of transmission.

**Sero logic procedures.** The complement fixation (CF) test was conducted in the standard manner at the United States Department of Agriculture Animal Disease and Parasite Research Laboratory, Beltsville. Serum samples from deer and calves were frozen immediately after collection and maintained in this state during shipment to the testing laboratory. Serum samples positive at a 1/5 serum dilution to the CF test were titrated.

The capillary tube agglutination (CA) test was conducted with commercial antigen* in a manner consistent with the recommendations of the manufacturer. The CA testing was performed within a few hours after serum samples were obtained and the serum was not frozen.

The card agglutination (Card) test was performed with antigen supplied by the United States Department of Agriculture. Both deer and calf samples were tested according to the procedure recommended for cattle. The Card test was conducted within a few hours after samples were obtained using plasma separated from blood containing the heparin anticoagulant.

**Hematological methods.** Anaplasma body counts were made on blood smears stained by the Giemsa method. Several hundred red blood cells in numerous microscopic fields were examined to determine the percentage which contained organisms.

**RESULTS**

Blood from 41 deer was inoculated into individual test calves. The results as shown in Table 2 indicate that 23 of the 41 deer were infected with *A. marginale*. The primary criterion for designating a deer as infected was the demonstration of Anaplasma bodies in the erythrocytes of its respective test calf. In each instance where Anaplasma parasitemia occurred, there was also the development of specific antibodies as measured by one or more of the serologic tests. Three of the calves (donor deer 926, 899, 897) developed antibody levels detectable on all 3 serologic tests but Anaplasma bodies could not be demonstrated in blood smears. Two of these calves were considered infected in spite of this deficiency. A splenectomy was performed on the third calf (donor deer 926) and Anaplasma bodies were detected 12 days later to confirm existence of the infection.

The interval between inoculation of intact calves with deer origin *A. marginale* and the appearance of marginal bodies in blood smears varied from 20 to 107 days. The interval between inoculation of calves and the appearance of first detectable antibodies varied from 20 to 91 days.

When the prevalence of infection was compared with the age of the deer (Table

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* Diamond Laboratories, Des Moines, Iowa
Anaplasma infection was closely associated with increased age. In fawns less than a year of age, 4 of 13 were infected with a prevalence rate of 31%. Yearling deer which had undergone one complete vector cycle had a prevalence rate of 47% with 7 of 15 animals proved to be Anaplasma carriers. In adult deer aged 2 years or more, only one of 13 animals was uninfected for a 92% prevalence rate.

These results would suggest that Anaplasma infection in the California black-tailed deer population is accumulative and that, once infected, an animal most likely remains a carrier the rest of its life. Of the 8 deer collected on the ranch were anaplasmosis was enzootic, one of 3 yearlings and 4 of 5 adults were infected. Although this sampling was small, it would appear that deer in close association with cattle do not necessarily have a higher prevalence of Anaplasma infection than non-cattle associated deer.

Serum from each deer was tested for the presence of specific antibodies using the CF and CA tests while plasma was used to conduct the Card test. The results of calf inoculation were used as a standard to which each of these serologic test methods was compared. There was a wide divergence in test results (Table 3), with few instances where all 3 tests were in agreement. In the testing of 41 deer, there were only 2 instances where all 3 tests identified infected deer and 9 where they identified negative deer.

The accuracy of each test as compared to the calf inoculation standard is shown in Table 4. The CF test was the least accurate with 20 correct, 18 false negative and 3 false positive results. The Card test was in the intermediate position with 24 correct, 12 false negative and 5 false positive results. The CA test, like the other 2 tests, was characterized by false negative reactions but it had only a single false positive test. With the CA test, there were 30 correct, 10 false negative and one false positive tests.

The antibody response of calves to *A. marginale* of deer origin was measured each week, beginning 14 days after inoculation and continuing until transmission was demonstrated or until animals had gone at least 90 days without evidence of transmission. All samples collected from calves 14 days after inoculation with deer blood were negative to the CF, CA and Card tests.

The CF test (Table 2) gave uniform and consistent results in that there were no false positive reactions and in only 4 instances were there false negative results (donor deer 8586, 926). After CF antibodies were once demonstrated in a calf, the weekly serum samples were positive as long as the calf was tested, except for the 2 instances noted above. In the 23 infected calves, antibodies were detected by one of the other test methods before they were found with the CF test in 3 instances (donor deer 8586, 927, 945).

The CA test results (Table 2) with calf sera closely paralleled those of the CF test. There were, however, 5 serum samples with which the CA test gave false positive reactions. If a false negative reaction was defined as that instance where negative CA and positive CF results were obtained with a serum sample from a calf which previously had one or more positive CA tests, then 13 CA tests could be classified as false negative. A good example is the test calf of deer 8585 which had 2 weeks of positive CA tests followed by 2 weeks of negative tests, then 4 positive tests and then a negative test.

The time of appearance of the first CA antibodies was similar to that of the
appearance of CF antibodies except in those cases where the calves had prolonged incubation periods. The test calf of deer 926 is a notable example. The first positive CF test occurred at 28 days post inoculation while the first positive CA test was at 98 days.

Results of the Card test (Table 2) were not as uniform and consistent as those of the CF and CA test methods. There were 6 false positive Card test reactions. If a false negative reaction was defined as that instance where negative Card and positive CF results were obtained with a sample from a calf which previously had one or more positive Card tests, then 18 Card tests could be classified as false negative. Such a system of determining false negative tests completely overlooks those 2 test calves (donor deer 8585, 911) that were Card negative during the entire course of their infection. The time of the first appearance of Card antibodies averaged about a week later than that of the CF and CA antibodies.

DISCUSSION

The results of this study confirm and supplement the previous reports of the high prevalence of the Anaplasma carrier state in black-tailed deer. The extensive exposure of deer to Anaplasma infection was shown with a 31% prevalence rate in fawns, a 47% rate in yearling deer and a 92% rate in adult deer. There was convincing evidence that deer in their natural habitat and not associated with cattle become infected and thus constitute a persistent reservoir of anaplasmosis.

California has a deer population estimated at approximately 1,500,000. These deer occupy public and private range lands which are used for the grazing of beef cattle. In California and other parts of the west where anaplasmosis is considered to be enzootic in the cattle population, both cattle and deer are parasitized by ticks known to be capable of transmitting Anaplasma infection. The recognition of deer as a true reservoir of infection identifies the most difficult obstacle in the way of eradicating anaplasmosis in cattle. The esthetic and sporting importance of deer to the people of California make it unlikely that measures will be taken to reduce the deer population to aid in the control of anaplasmosis in cattle.

A reliable serologic test applicable to deer and other wild ruminant species is needed to determine the extent of the wildlife reservoir of anaplasmosis. In one study, the CF and CA tests gave inconsistent results with sera from experimentally infected mule deer. In another report of induced *A. marginale* infections in splenectomized (*Odocoileus virginianus*) deer, there was a close relationship between parasitemia and CF serum titers. In the same study, the CF test was 56% accurate with known negative sera and 98% accurate with known positive sera while the CA test was 100% accurate with known negative sera and 94% accurate with known positive sera. Antibody titers persisted in these white-tailed deer for 112 days after inoculation.

Reports of experimental infections of black-tailed deer were in contrast to experimental anaplasmosis in white-tailed deer. When 4 adult non-splenectomized black-tailed deer were inoculated with blood from a bovine carrier, all produced antibodies but titers were not high and sera tested 112 days after infection were negative to the CF test. Eleven months later, 3 of these same animals were still Anaplasma carriers and again were negative to the CF test. Splenectomized
black-tailed fawns also had an antibody response to infection, but the titers rapidly receded below a detectable level in surviving animals. In the 2 reports of serologic testing of naturally infected black-tailed deer, sera from both infected and non-infected deer were negative to the CF test.

In the present study, the CF, CA and Card tests were applied to the serum or plasma of naturally infected black-tailed deer with each test method being conducted in the manner recommended for cattle samples. Each test, and particularly the CF test, was plagued by false negative reactions. False negative results were most common in the young fawns which presumably were in the early stages of infection and in the adult deer with long-standing infections. Best results were obtained with samples from yearling deer where the CA test had an overall accuracy of 94%. It is quite probable that modifications could be made in the CA and Card tests that would lessen the number of false negative serologic reactions found in adult deer with long-term infections. If this were accomplished, it would be possible to conduct meaningful surveys of the prevalence of anaplasmosis in deer without having to rely on the costly calf inoculation procedure used in this study.

The CF, CA and Card tests were compared using samples collected during the prepatent and patent phases of anaplasmosis in 23 intact calves. These same tests were also compared using samples collected from 18 non-infected calves during a 12-week period. In this study where the demonstration of Anaplasma bodies in erythrocytes of calves was the primary criterion used to confirm the presence of infection, the CF test proved to be a highly accurate diagnostic procedure even in those cases where the calves had prolonged incubation periods. Comparable results were obtained with the CA test except in those calves with the prolonged incubation periods. It was not determined whether the inability to obtain positive CA test results in these cases was due to the absence of agglutinating antibodies or to a deficiency in the test itself. Refinements or changes will be necessary before the Card test can match the accuracy of the CF and CA tests when performed on calves during the prepatent and patent phases of anaplasmosis.

REFERENCES

# TABLE 1

Resume of Published Data of Previous Surveys of the Anaplasma Carrier State in California Deer

<table>
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<tr>
<th>No. of Deer</th>
<th>Age of Deer</th>
<th>Transmission Results</th>
<th>Area of Collection</th>
<th>Date of Collection</th>
<th>Literature Reference</th>
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<td>Boynton and Woods¹²</td>
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<td>18</td>
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<td>Mendocino</td>
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</tr>
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TABLE 2. Results of a Calf Inoculation Study in Which One Intact Calf Was Used for Each of 41 Columbia Black-tailed Deer Tested for the Anaplasma Carrier State.

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<th>Infection Demonstrated</th>
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<td>36</td>
<td>38</td>
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</tbody>
</table>

-- = Sample tested and results negative
ND = Not done
* = Blood was obtained from calves 3 times per week for Anaplasma body detection and once per week for serologic studies.
** = Day when positive test result was obtained
*** = The spleen had been removed from this calf 12 days before demonstration of Anaplasma bodies.
## Table 3
Correlation of Anaplasmosis Serologic Tests with the Results of Calf Inoculation on Blood Samples Collected from 41 Columbian Black-tailed Deer.

<table>
<thead>
<tr>
<th>No.</th>
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<th>CF***</th>
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<td>-</td>
<td>+</td>
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<td>5 mo.</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>5 mo.</td>
<td>+</td>
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<td>-</td>
<td>-</td>
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<td>Pos.</td>
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<td>4 yr.</td>
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<td>10 yr.</td>
<td>-</td>
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<td>Pos.</td>
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* Card test  
** Capillary Agglutination test  
*** Complement Fixation test
A. MARGINALE INFECTION IN CALIFORNIA DEER

TABLE 4

Correlation of Serologic Tests with Results of Calf Inoculation for 41 Deer Blood Samples Checked for the Anaplasma Carrier State.

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<tr>
<th>Test</th>
<th>Agreement with Calf Inoculation</th>
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<th>False Positive</th>
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<td>30</td>
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REPORT OF THE ANAPLASMOSIS COMMITTEE

J. W. Safford, Chairman, Helena, Montana

The Anaplasmosis Committee met the afternoon of October 13, 1969 and October 14, 1969 in Milwaukee, Wisconsin.

The following papers were presented at the committee meetings and will be included in the proceedings:

FOREST HENDERSON
“Pilot Programs to Control Anaplasmosis in Louisiana” — A Preliminary Report.
T. E. AMERAULT and T. O. ROBY
“Current Laboratory and Field Studies on the Anaplasmosis Card Test”.
K. L. KUTTLER
“Serial Passage of an Attenuated Anaplasma marginale in Calves”.
J. A. HOWARTH, T. O. ROBY and D. W. McNEAL
“Prevalence of Anaplasma Infection in California Deer as Measured by Calf Inoculation and Serologic Techniques”.
C. J. WELTER and M. RISTIC
“Laboratory and Field Trials with an Attenuated Anaplasma marginale Vaccine”.

In addition to the recent research work reported in the papers presented to the committee, the research work accomplished in developing serological tests, vaccines and therapy the past few years, as reported in previous committee reports, was reviewed. The committee recommends that emphasis be placed on the following areas of research:

1. Continued research on the testing and evaluation of new therapeutic agents.
2. Increased research and investigation into vector transmission of anaplasmosis and detailed epidemiological studies in varied geographical areas.
3. Continued evaluation of the card agglutination test.

The committee recognizes the need for valid information concerning the incidence and geographical distribution of anaplasmosis in the United States and recommends that all states conduct serological surveys using available MCT blood samples.

An anaplasmosis pilot control program is being conducted in Louisiana. Recognizing the differences in climate, terrain, vectors and wild-life populations, the committee recommends that additional pilot projects be initiated in other distinctly different geographical areas of the United States.
PROGRESS OF THE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM

By H. C. King

Progress in eradicating brucellosis during fiscal year 1969 has been substantial. However, as was mentioned last year, time is getting short and the rate of progress must be increased to reach the 1975 Certified Brucellosis-Free goal. It is much more costly to prolong eradication than to conduct an intensive program that would bring about the desired results in a short period of time. Unless eradication is achieved by 1975, it will be extremely difficult to continue the program beyond that date at effective levels. It is, therefore, extremely important that the program be conducted at a maximum level in all areas of the country to achieve this goal.

During fiscal year 1969, two States — Mississippi and Oklahoma — qualified as Modified Certified Brucellosis Areas compared with three States which qualified during fiscal year 1968. Six States — Florida, Hawaii, Louisiana, Nebraska, South Dakota, and Texas — had not achieved Modified Certified Brucellosis Area status at the close of the fiscal year; however, Hawaii qualified in September. A year ago, eight States were in this category; a reduction of only two during the fiscal year.

Tangible progress made during the last fiscal year can also be measured by the number of States reaching a Certified Brucellosis-Free status. Three States — Arizona, California, and New Jersey — reached this goal bringing the total to 15 States plus the Virgin Islands. On June 30, 1969, Utah lost its statewide certification, but requalified in September 1969. Since the end of fiscal year 1969, Delaware and West Virginia qualified as Certified Brucellosis-Free, bringing the current total to 18 (fig. 1 & 2).

With a third of the States now certified as Brucellosis-free, increased efforts and activities will be required to qualify 32 States and Puerto Rico in the next 6 years.

During fiscal year 1969 there was a net increase of 81 counties in the total qualified as Certified Brucellosis Areas. These 3,038 certified counties represent 96 percent of the 3,153 counties in the United States, Puerto Rico, and the Virgin Islands. By the end of the fiscal year, 1,713 counties (54 percent of the total) were Modified Certified Brucellosis Areas, and 1,325 counties (or 42 percent) had reached a Certified Brucellosis-Free status. The number of counties not modified certified was reduced to 4 percent compared to the 6 percent in fiscal year 1968 (fig. 3).

At the end of 1968, only 36 percent of the counties had reached free status. The 42 percent free in 1969 appears to be significant progress, but only 205 counties qualified for free status in 1969 compared to 258 counties in 1968.

A total of 18,020 infected herds were reported in fiscal year 1969, a reduction of 2,643 herds compared to the 1968 total. In 1969, 152 infected herds were found in the 15 Certified Brucellosis-Free States; 157 herds were reported in 1968 in 13

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free States. No infected herds were found in three free States — Connecticut, New Hampshire, and New Jersey — and five or less infected herds were found in each of five other free States.

In the 29 modified certified States, 7,621 infected herds were found, approximately 1,300 more than were reported in 1968. Twenty or less infected herds were found in each of eight modified certified States. Two States — Oklahoma and Mississippi — accounted for over 46 percent of the infected herds as a result of concentrated efforts during the year to qualify as modified certified States.

Almost 60 percent of the infected herds were found in the six States not modified certified. Over 90 percent of these infected herds were found in Texas and Louisiana where over 64,000 herds were tested in efforts to qualify counties (fig. 4).

There was little change from previous years in the percentage of herds that revealed reactors on only a single test in the Brucellosis-free counties. Of all the reactors in free counties, 62 percent had only a singleton reactor, 8 percent had two reactors per herd on a single test, and 30 percent had reactors on more than one test.

The number of free counties in which reactor herds were found increased from 495 to 675. Of concern is the increased number and percentage of counties revealing infected herds with multiple reactors. The number of these counties increased from 228 in 1968 to 313 in 1969 (20 percent to 24 percent).

Greater efforts must be made to find infected herds and to eliminate the infection before it has a chance to spread within the herd and to other herds. Epidemiological capabilities have been increased during recent years; they should be used to the fullest extent to quickly locate and clean up infected herds.

The total number of blood tests made last year declined by over 1 million head compared to the previous year. The decrease occurred in farm tests, primarily as a result of reductions in MCT reactors and suspicious BRT’s requiring followup herd tests. Market cattle tests accounted for 50 percent of the 10.8 million total tests performed in 1969 compared to 40 percent of the total tests in 1968. Total reactors decreased by 19,000 to approximately 130,000 (fig. 5).

No significant change has taken place during the past 4 years in coverage under the market cattle testing program. The level of coverage during the past year is almost identical to that in 1966 although reflecting a slight increase over 1967 and 1968.

Almost 5 million blood samples were collected from cows and bulls in market channels. A total of 2.9 million of these were collected at slaughter plants and almost 2 million at markets and other points (fig. 6).

Followup herd tests were made on 10,812 herds as a result of 31,595 MCT reactors found. Of these herds, 3,811 were found to be infected on the initial herd test. There were 21,774 reactors found on the first test of these herds representing 12.9 percent of all eligible cattle in the infected herds. The high rate of infection found indicates a failure, in many instances, to locate these herds until the infection has become widespread. This rate is almost three times that of dairy herds found infected as a result of followup BRT suspicious herd tests.

Market cattle testing coverage and traceback capability must be increased if
this procedure is to be reliable for locating infected herds. As the incidence of the disease decreases, it becomes increasingly important that surveillance procedures become more effective if they are to be relied upon to locate the remaining reservoirs of infection.

Another measurement of the effectiveness of the MCT program is the percentage of animals tagged with the white side of the tag showing from which blood samples are collected. This past year blood samples were collected from only 57 percent of over 6 million white-tagged cows. A wide range of recovery rates were reported by the individual States, ranging from a low of 15 percent to a high of 98 percent. In nine States, the recovery rate was reported to be above 75 percent, while in five States the rate was below 25 percent. The percent of backtagged reactors that could not be traced to herds of origin was 5.5 percent, approximately the same as a year ago.

Every means must be exerted to increase the application of backtags, to increase the percent of tagged animals that are tested, and to trace and test the herds of origin of all MCT reactors.

There were 1,394,011 Brucellosis milk ring tests (BRT’s) conducted this past year, almost 200,000 less than in 1968. Nationally, dairy herds are milk ring tested three to four times per year. The decline in total tests reflects, in general, a decreasing number of dairy herds in the Nation.

The percent of herds suspicious to the ring test continued at 0.4 percent, the same as last year (fig. 7). However, there was a wide variation between States fluctuating from a low of no suspicious herds to a high of over 18 percent in one State. In general, the rates reflect the brucellosis status of the States; in 18 States the rate was 0.2 percent or lower.

Almost 6,000 BRT’s were suspicious; as a result, 4,795 initial followup herd blood tests were made. These tests resulted in locating 985 infected herds or approximately one infected herd for every five suspicious BRT herds tested. There were 2,715 reactors found on the first test of these herds representing 4.2 percent of all eligible cattle in the herds. This important program tool has proven its effectiveness in locating infected dairy herds. However, continuous review must be made of the procedures used and corrections made as necessary in order to maintain its effectiveness.

Calf vaccinations declined for the fifth consecutive year. The decrease of over 750,000 calves is 12 percent less than the number vaccinated in 1968. This is the largest annual decline that has occurred since the peak of vaccination was reached in 1964 (fig. 8). Decreases occurred in all but five States; and in these the increases were minimal and averaged less than 2,500 calves each.

Over one-fourth of the 5.3 million calves vaccinated were in Certified Brucellosis-Free States. There is little justification for the continued use of Strain 19 in free States. Vaccination in areas which have minimal exposure possibilities can only result in diagnostic problems and in animals which fail to overcome the Strain 19 infection. During a 9-month period from December 1968 through August 1969, 15 of 60 reported Brucella isolations from vaccinated animals were typed as Strain 19. In five Certified Brucellosis-Free States, in which isolations were made, 13 of 21 isolations were typed as Strain 19.

The number of lots of swine tested in fiscal year 1969 decreased to 60,904, a
decrease of about 20 percent. Part of the decrease can be attributed to completion of the pilot project in Iowa early in the year. The infection rate continued to decline with only 1.3 percent revealed this year as compared to 1.8 percent last year (fig. 9).

One additional State — Arizona — qualified as Validated Brucellosis-Free bringing the total to four States and the Virgin Islands. County validation increased during the year from 154 counties to 182, an increase of 28. In addition to all the counties in Arizona, Nevada, Utah, Vermont, and the Virgin Islands, there are 52 validated counties in California, two in Connecticut, two in Hawaii, one in Maryland, eight in Massachusetts, two in Tennessee, and 35 in Puerto Rico (fig. 10).

The number of Validated Brucellosis-Free swine herds increased during the year by approximately 700 to make a total of over 3,000. Validated herds are located in 45 States and Puerto Rico.

During the past year 17 States were engaged in some type of a market swine testing program (MST). Many of these programs are of limited extent at this time but are designed to evaluate various identification and blood collection procedures. All States are encouraged to initiate and expand the MST program.

The Iowa pilot project to study the problems associated with swine identification, the collection of blood samples at slaughter, and the use of the brucellosis card test as a diagnostic procedure for samples collected at slaughter, was completed during the past fiscal year. Over 18,000 heart clot blood samples were collected from sows slaughtered at the Farmbest Plant in Iowa. Most of these sows were identified by a slap tattoo when delivered at the plant's buying stations.

The card test revealed 30 positive animals from nine herds as compared to 169 animals from 130 herds which were positive at the 1:50 dilution or higher on the plate agglutination test. Serological studies and culture attempts failed to indicate infection in herds from which marketed sows were found to be negative to the card test but which revealed titers on the plate agglutination test. The following conclusions may be made:

1. The card test is an effective method for detecting brucella infected swine herds.
2. The slap tattoo was effective in identifying hogs purchased at buying stations. However, only four of nine lots of sows found positive on the card test could be identified to herds of origin because five lots had been purchased from commission companies.
3. Blood samples collected from the heart clot, although not of the best quality, are adequate for card testing under a market swine testing program.

The Brucella abortus 45/20 vaccine study conducted jointly by Clemson University and the Animal Health Division is continuing. Serological responses of cattle vaccinated with this vaccine are being recorded and studied. The first annual revaccination, as recommended by the European producers of the vaccine, was completed in November 1968. The cattle are scheduled to be challenged in January 1970. Until all studies are completed, it would be unwarranted to draw conclusions. A full report should be available for this organization at its next annual meeting.

In summary, a review should be made of how well the previously established goals for eradicating brucellosis have been met. At the beginning of the last fiscal
year, a compilation of jointly established goals indicated that an additional 365 counties would achieve a Certified Brucellosis-Free status during the year. Actual achievement fell 160 counties short of this goal with only 1,325 Certified Brucellosis-Free Counties at year’s end.

It had been projected that only 87 noncertified counties would remain by the end of the year and that area testing would be underway in all of these. However, 115 counties were still not modified certified as of July 1, 1969, and nine of these counties had failed to establish organized area work (fig. 11).

Failure of States to meet established intermediate realistic goals will jeopardize the chances of reaching the Nation’s goal as Certified Brucellosis-Free by 1975. Projections for the fiscal year 1970 are that 209 counties will achieve Certified Brucellosis-Free status bringing the total to 1,534. In addition, all remaining counties will be modified certified with the exception of 78 counties in which organized Brucellosis eradication programs will be operating.

These goals for eradicating Brucellosis are realistic and well within the available resources of funds, knowledge, and manpower. The most efficient use of these resources must be made while they are still available.
BRUCELLOSIS ERADICATION PROGRAM
NONCERTIFIED AREAS
SEPTEMBER 30, 1969

Brucellosis Eradication
COUNTY CERTIFICATION STATUS
June 30, 1968
June 30, 1969

Percent of U.S. Counties
- Certified Free
- Area Work in Progress
- Modified Certified
- Individual Herd Participation

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
BRUCELLOSIS INFECTED HERDS FOUND
In Noncertified, Modified Certified and Certified-Free States

NUMBER INFECTED HERDS

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FISCAL YEAR


Biological Testing: Cattle

MILLIONS CATTLE TESTED

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THOUS. REACTORS FOUND

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## Brucellosis Eradication

### MARKET CATTLE TESTING PROGRAM

**Cows Blood Tested**

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### MILK RING TESTING: HERD TESTS

- **27.0%** in 1952
- **0.4% (1969)**
**Brucellosis Eradication**

**CALVES VACCINATED**

MILLION CALVES VACCINATED

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<td>4</td>
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<td>6</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
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</table>

**BLOOD TESTING: SWINE**

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Herds-Lots Tested</td>
<td>14,919</td>
<td>59,328</td>
<td>61,372</td>
<td>13,769</td>
<td>60,904</td>
</tr>
<tr>
<td>Infected Herds-Lots</td>
<td>31%</td>
<td>24%</td>
<td>18%</td>
<td>13%</td>
<td></td>
</tr>
</tbody>
</table>

U.S. Department of Agriculture
Animal Health Division
Agricultural Research Service
PERSISTENCE OF BRUCELLA ABORTUS, STRAIN 19
INFECTION IN IMMUNIZED CATTLE

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Claude J. Nelson D.V.M., M.P.H.
United States Department of Agriculture
Agricultural Research Service

In spite of continuous and strenuous research efforts by many investigators, no strain of Brucella, method of organism inactivation or protocol of injection yet has been found that endows to killed, virulent Brucella the ability to stimulate in cattle immunity comparable in level or duration to the immunity produced by live, avirulent organism. The immunizing agent used to protect cattle against brucellosis, B. abortus, strain 19, is markedly less virulent and less able to invade tissues than are other strains of this species. Hence, even though the organisms are capable of reproduction after administration to an animal, most heifers given an immunizing dose of B. abortus, strain 19 during calfhood are well able to clear their tissues of infection and reach immune status prior to reaching sexual maturity. Some few animals, however, cannot free themselves of the organisms and such animals enter maturity with Brucella infection rather than with immunity to the disease. In a dairy herd, the presence of animals residually infected with the immunizing organism usually is first detected by reversion of the Brucella Milk Ring Test (BRT) from a negative reaction to a suspicious reaction. The subsequent course of events is to identify the infected individuals by the combined use of serum agglutination and supplemental tests and then consign the infected animals to slaughter. By regulation, regardless of the infecting strain of organism, reactor cattle in control areas have been slaughtered and, consequently, there have been no data to characterize residual, localized infections of B. abortus, strain 19 in mature cattle. The purpose of this investigation was to ascertain the duration of residual infections resulting from immunization and to determine the effects of infection on gestation periods and viability of the offspring and on duration and fluctuation of antibody titer. In order to conduct this type of study, the course of events following the identification of an infected animal was altered. The milk of animals have positive reactions to the supplement tests was cultured and animals found to be excreting B. abortus, strain 19 were purchased by the United States Department of Agriculture and were maintained by the School of Veterinary Medicine, University of California at Davis. The results of a three-year surveillance of these animals are reported herein.

Materials and Methods

The 10 herds from which an eventual total of 11 animals were purchased were

* This investigation was supported in part by Public Health Service Research Career Program Award Ke-A1-19,309 and by Grant A1-4638, both from the National Institute of Allergy and Infectious Diseases.
located in either Modified or Certified Brucellosis-Free counties in California. All of the herds had shown negative BRT tests for continuous periods ranging from 1 to 9 years and had then reverted to displaying a suspicious reaction. Serum testing of individual animals by the standard tube test almost always failed to reveal agglutination titers sufficiently high to classify any animal as a reactor. Consequently, the herds were designated as problem herds, each herd was segmented and the segments BRT tested. In each herd, one segment had a suspicious reaction to the BRT test and each animal within this segment then was examined for its reaction to the standard serum tube agglutination, whey plate agglutination, Rivanol and acidified plate antigen tests. Milk samples from animals that had positive reactions to the supplemental tests were obtained for culture. In each of these 10 herds there was only one offending animal in the suspicious segment although one of the herds had a second infected animal at a later date.

*Brucella* organisms were isolated from each of the 11 milk samples. By combined use of conventional and manometric determinative procedures²,³, the isolates were identified as *Brucella abortus*, strain 19. Once it had been established that an animal was shedding *B. abortus*, strain 19 via the mammary gland, she was purchased for this project. The first animal included in the study (no. 101) was obtained in January, 1966. The last animal (no. 111) was added to the group in April 1967. All of the animals were consigned to slaughter simultaneously in January, 1969.

The history of each animal at the time of purchase is presented in Table 1. Eight of the animals either were pregnant on arrival or were bred shortly after their arrival and calved during the course of this project. Three non-pregnant animals were in early lactation and nurse calves were purchased for them. Both the cows and calves were tested every 45-60 days by the standard tube agglutination and supplemental serum tests. Simultaneously, quarter milk samples from each lactating animal were cultured and also examined by the whey plate agglutination test. Dry secretions were cultured from non-lactating animals. At parturition the fetal membranes were cultured.

**Results**

Table 2 shows the bimonthly serum antibody titers of 11 heifers residually infected from immunization with *B. abortus*, strain 19. The following information can be derived from this table: (1) At the time of initial reversion of the BRT test in the herds of origin, only 1 heifer (no. 102) had a sufficiently high serum antibody titer to be classified as an official reactor. (2) Each animal had fluctuations in its serum antibody titer response to residual infection with *B. abortus*, strain 19 but, except for the characteristic of fluctuation, there was no pattern or over-all uniformity of the response. Shortly after purchase, four of the animals (101, 102, 103, and 104) reached and rather persistently maintained reactor titers; animal 107 reached reactor status for only a brief and fleeting interval; animal 108 reached, and animal 110 approached, reactor status almost two years after the initial isolation of *B. abortus*, strain 19 from their milk; and three animals (105, 109, and 111) did not have a titer in the reactor classification during the course of this study.
Results of the supplemental tests on the heifers. Each animal had a positive reaction to the card test at each testing period. Except for a brief period for two of the animals, the Rivanol test also was uniformly positive. In May and again in July, 1968, animals 110 and 111 both had negative reactions to the Rivanol test.

Concurrent with the taking of blood samples, quarter milk samples were obtained from each lactating animal. In each instance, each animal gave a positive reaction to the whey plate test in the milk from at least one quarter and frequently from two quarters. Milk from the positive quarters was cultured and also usually inoculated into guinea pigs. Brucella abortus, strain 19 was recovered intermittently from each animal during her 1½ to 3 year surveillance. More precisely, each animal was excreting Brucella in her milk when she was brought into the project; 9 of the 11 animals still were excreting it at the termination of the project; and during the 3 years spanned by the study, B. abortus, strain 19 was isolated at least 3 times from each animal. The maximum number of isolations was 8, from animal no. 101.

Results from culturing fetal membranes and fluids. During the course of this project, the 8 animals that had been bred, each delivered a viable, full-term calf. No Brucella organisms were recovered from fetal membranes, fluids, or vaginal swabs on either direct culture or from inoculated guinea pigs.

Results of serum testing the calves. One calf, though full term, died 8 days after birth. No organisms, Brucella or otherwise, were recovered from cultures of blood and internal organs and tissues, nor was there any gross or microscopic pathology and the cause of death remains unknown.

The 7 other calves born during the project remained healthy, as did the nurse calves. At the termination of the project, the calves ranged in age from several months to 1½ years. The blood serum on all the calves consistently remained negative to all the tests.

DISCUSSION

Residual infection with B. abortus, strain 19 in mature heifers commonly is attributed to having immunized the animals over the officially recommended age limit. However, 6 of the 11 residually infected heifers included in this investigation were immunized at 8 months of age and under. Obviously, residual localization of the organisms following immunization is not entirely attributable to overage vaccination.

In animals infected consequentially from immunization, age at the time of immunization apparently does not later influence either the fluctuations or magnitude of the serum antibody titer as measured by the standard tube agglutination test. Infected animals immunized within the age limits can show a reactor titer of long duration (no. 101) and animals immunized over age can show persistently low serum tube agglutination titers (no. 111).

Features common to each of these heifers include a continuously positive card test and whey test, an essentially continuously positive Rivanol test, and chronic, intermittent shedding of B. abortus, strain 19 in their milk. It seems clear, therefore, that when infection persists over the normal post-immunization recovery period the animals cannot later clear their tissues of the organisms and they remain residually infected for long periods of time, perhaps permanently. However, even
though these 11 heifers were intermittently shedding *B. abortus*, strain 19 in their milk, nursing calves in confinement with the heifers showed no titer response after up to 1½ years. This time period represents more than the normal cow-calf relationship under natural conditions.

Residual *B. abortus*, strain 19 infection in mature cattle can be further characterized by the fact that, in these 11 heifers, it did not spread to or involve the reproductive tract. Three of the heifers each had had a normal gestation period and parturition and delivered full term normal offspring shortly before their inclusion in the project. Eight had normal gestations, parturitions and viable offspring during the study. Apparently, *B. abortus*, strain 19 generally does not residually localize in the reproductive tracts of mature heifers and cause abortion in these animals. However, heifers maintaining a post-immunization infection into sexual maturity remain chronically infected and excrete the organisms via the mammary gland for long periods of time. Obviously, these animals should be regarded as any other reactor and consigned to slaughter. Animals that remain residually infected as a result of immunizations are few in number. From 1963 through 1969, only 21 such animals were identified within the State of California but during this time over 1,575,000 dairy calves were immunized with *B. abortus*, strain 19. Thus, another characteristic of residual infection of *B. abortus*, strain 19 is its remarkably infrequent occurrence.
TABLE 1

Immunization and Breeding History of Eleven Heifers With Residual
*Brucella abortus*, strain 19 Infection

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Date Purchased</th>
<th>Age At Purchase</th>
<th>Breed</th>
<th>Age At Vaccination</th>
<th>Age At First Calving</th>
<th>Serum Titer</th>
<th>Status At Purchase</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>July 1966</td>
<td>32 mo.</td>
<td>Jersey</td>
<td>8 mo.</td>
<td>24 mo.</td>
<td>i 1:200</td>
<td>Susp.</td>
</tr>
<tr>
<td>104</td>
<td>Dec. 1966</td>
<td>30 mo.</td>
<td>Jersey</td>
<td>15 mo.</td>
<td>30 mo.</td>
<td>i 1:100</td>
<td>Susp.</td>
</tr>
<tr>
<td>105</td>
<td>Jan. 1967</td>
<td>30 mo.</td>
<td>Jersey</td>
<td>8 mo.</td>
<td>20 mo.</td>
<td>1:100</td>
<td>Susp.</td>
</tr>
<tr>
<td>106</td>
<td>Aug. 1966</td>
<td>5 yrs.</td>
<td>Guernsey</td>
<td>6 mo.</td>
<td>33 mo.</td>
<td>i 1:50</td>
<td>Neg.</td>
</tr>
<tr>
<td>107</td>
<td>Feb. 1967</td>
<td>32 mo.</td>
<td>Jersey</td>
<td>6 mo.</td>
<td>33 mo.</td>
<td>i 1:50</td>
<td>Neg.</td>
</tr>
<tr>
<td>110</td>
<td>Apr. 1967</td>
<td>48 mo.</td>
<td>Jersey</td>
<td>12 mo.</td>
<td>29 mo.</td>
<td>i 1:50</td>
<td>Neg.</td>
</tr>
</tbody>
</table>
# TABLE 2

Bimonthly Serum Agglutination Titers on Eleven Heifers Residually Infected With *Brucella abortus*, Strain 19

<table>
<thead>
<tr>
<th>Yr.</th>
<th>Month</th>
<th>Animal Number and Serum Agglutination Titer by Tube Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>101&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>1966</td>
<td>Jan.</td>
<td>i1:200</td>
</tr>
<tr>
<td></td>
<td>March</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>Sept.</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>1:50</td>
</tr>
<tr>
<td>1967</td>
<td>Jan.</td>
<td>i1:100</td>
</tr>
<tr>
<td></td>
<td>March</td>
<td>1:200</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>1:200</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>1:200</td>
</tr>
<tr>
<td></td>
<td>Sept.</td>
<td>i1:100</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>i1:100</td>
</tr>
<tr>
<td>1968</td>
<td>Jan.</td>
<td>1:100</td>
</tr>
<tr>
<td></td>
<td>March</td>
<td>i1:200</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>i1:100</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>Sept.</td>
<td>i1:100</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>1:200</td>
</tr>
<tr>
<td>1969</td>
<td>Jan.</td>
<td>1:200</td>
</tr>
</tbody>
</table>

<sup>1</sup>over officially recommended age at time of immunization  
<sup>2</sup>consigned to slaughter April 1967 because of severe pneumonia
REFERENCES


REPORT OF THE COMMITTEE ON BRUCELLOSIS

United States Animal Health Association
Seventy-Third Annual Meeting
October 12-17, 1969

H. G. Wixom, Sacramento, California, Chairman

Your Brucellosis Committee met in open session on Tuesday afternoon and gave opportunity for anyone to present their recommendations or ideas pertaining to the National Brucellosis Eradication Program. The Committee heard progress and special reports and explored all items that came to their attention to improve or make the program more efficient and to make sure that the program was on course to achieve previously stated objectives. The sessions were interesting and constructive. However, they were noticeably free of objections which have been evident in previous sessions. We trust this reflects that the program is reasonably well worked out and that our goal of complete eradication of brucellosis is coming into view and will be achieved.

Progress. Four more entire states have achieved a Certified Brucellosis-Free status bringing the total to 18. One hundred ninety-five counties have been added to the Free list. Significant progress is noted in accomplishing Modified Certified Brucellosis area. Eighty-two additional counties and two states—Mississippi and Oklahoma—were modified since last year. Now only five states remain as not Modified Certified. Good progress is being made in each of these states and the Committee is encouraged to be informed that all states will be at least Modified Certified within the near future if present progress continues.

Interstate Regulations. On August 1, 1969, the Federal Interstate Regulations with respect to brucellosis were amended in accordance with the recommendations of the U. S. Animal Health Association last year and in previous years. The U. S. Department of Agriculture is commended for taking this important action to protect the gains made by the various states and to prevent further spread of brucellosis from infected areas. To be effective, these regulations must be vigorously enforced. Therefore, we recommend that the U. S. Department of Agriculture apply adequate enforcement to insure uniform compliance with the regulations by all industry members.

On September 20, 1969, the U. S. Department of Agriculture amended the Code of Federal Regulations making official vaccination ages in conformity with the Uniform Methods and Rules for Brucellosis Eradication as recommended by the
U.S. Animal Health Association in its Brucellosis Committee report last year.

**Testing of Official Vaccinates.** Last year your Committee recommended that the Uniform Methods and Rules and the Federal Interstate Regulations require:

"Beginning January 1, 1970, officially vaccinated heifers of the beef breeds be tested at 24 months of age, and those of the dairy breeds at 20 months of age."

This recommendation has been carried out in the Uniform Methods and Rules, but is yet to be implemented in the Federal Interstate Regulations. The Committee has again re-evaluated this proposal and reaffirm our recommendation.

**Bison.** The Committee reaffirms its recommendation made in 1966 that a program for the eradication of brucellosis in bison be established and carried out equal to that applied to domestic cattle.

**Revised Recommended Uniform Methods and Rules.** Your Committee received a copy of the Recommended Uniform Methods and Rules for Brucellosis Eradication (ARS 91-79) revised as of September 1969 by the United States Department of Agriculture, Agricultural Research Service. This revised issue contains the recent recommendations of your Committee and has been completely rewritten for clarity. Those who have worked on this revision are to be commended.

**Card Test Film.** The Committee reviewed an advanced copy of a film illustrating the use of the card test for brucellosis. This film will be available for each state for instructional purposes and states are urged to use this excellent visual aid.

**Brucella melitensis.** A report was heard on an outbreak of *Brucella melitensis* in goats and sheep near the Mexican border in Texas. This outbreak was quickly eradicated and much valuable information on the testing of goats and sheep for brucellosis was obtained. This information can be applied in the event other outbreaks occur.

**Imports.** It has been brought to the attention of the Committee that in the near future, cattle might be imported into the United States under restrictions less than those for moving cattle interstate. Your Committee recommends that the U.S. Department of Agriculture establish import regulations equal to requirements for interstate commerce.

**Swine Validation.** The progress of validating brucellosis free areas continues to be slow. Currently there are 184 validated free counties. This includes all counties in four states and the Virgin Islands. The key to accelerating this phase of the eradication effort is in development of a swine identification program which will permit accurate traceback of reactor animals. The U.S. Department of Agriculture is encouraged to continue their search to find a satisfactory identification method which will permit expansion of market swine testing.

Public health officials are still expressing concern regarding brucellosis in man from swine sources. States are urged to develop a swine brucellosis program in harmony with recommendations previously made to eliminate this health hazard.

**Market Cattle Testing Program.** It is essential that the Market Cattle Testing program be further expanded as necessary to certify and recertify area under the eradication program. Concerted efforts should be made to obtain blood samples from backtagged cattle at slaughter through cooperation of all Meat Inspection Services and the packing industry.
Objective. In projecting our progress in eradicating brucellosis, it appears that we are currently on target to achieve eradication by 1975. To accomplish this, the tempo must not be slackened, but increased especially in areas that are not yet Modified Certified and in areas that recently achieved this status. Your Committee will continue to study the progress and stands ready to make recommendations if they be needed to accomplish this stated goal which is now well accepted throughout the nation.
EQUINE LEPTOSPIROSIS

L. E. Hanson, D.V.M., Ph.D.
R. J. Martin, D.V.M., M.P.H.
R. W. Gibbons, D.V.M.
P. R. Schnurrenberger, D.V.M., M.P.H.

Dr. Hanson is Head of the Department of Veterinary Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana. Dr. Martin and Dr. Schnurrenberger are public health veterinarians with the Illinois Department of Public Health, Springfield. Dr. Gibbons is with the United States Department of Agriculture, Animal Health Division.

Serologic surveys in many countries indicate leptospiral infections occur frequently in horses and can be caused by a variety of serotypes (Table 1). Similarly, leptospiral organisms have been isolated from horses worldwide (Table 2). While reactor rates of horse populations may be greater than for other livestock, clinical signs are usually less severe and extensive than in other species.

Leptospirosis is not generally accepted as a major problem of horses although reports indicate the disease has been responsible for serious losses in some herds. The clinical signs associated with equine leptospiral infections are quite variable. The acute form may be manifested as fever, depression or dullness, anorexia, icterus and neutrophilia. The most commonly recognized form of the disease in horses is the persistent condition, periodic ophthalmia.

Roberts, et al. (38) reported an acute outbreak of leptospirosis which occurred on a New York farm. Sixteen horses were involved, six of which exhibited clinical signs. Five of the horses showed signs of septicemia and one foaled two weeks prematurely. The foal was weak, unable to stand and icteric. Sera from six horses contained leptospiral CF antibodies; 4 of the 6 sera had titers of 1:32 to 1:512. *L. pomona* was isolated from guinea pigs inoculated with blood from two of the sick mares.

Jackson et al. (43) associated abortions in mares with leptospiral infections. The sera of 3 mares which had aborted near term contained *L. pomona* antibodies. Subsequent sera from these animals had higher agglutination titers. Serum from a fourth mare that aborted a 7½ month fetus titered 1:1000 against *L. pomona* on the day of the abortion.

Artificial infection studies using *L. pomona* have been reported by Heusser (44), Bürki et al. (45) and Morter et al. (46 & 47). These investigators reisolated *L. pomona* from horses that had developed signs of infection after inoculation of the organism.

The pathogenesis of periodic ophthalmia (recurrent iridocyclitis or “moonblindness”), of horses is quite complex. Although eye lesions may occur during the initial acute infection of leptospirosis, the lesions usually subside completely. The typical lesions of periodic ophthalmia apparently result from recurrent infections and are probably an allergic response to localized foreign or modified host antigens. The recurrent reactions may be produced by injections of other serotypes as well as the initial infecting serotype. The relationship of leptosprial antibodies to signs of
periodic ophthalmia has been reported by a number of workers (Table 3). Many serotypes have been associated with the condition. Heusser (44), Yager (50) and Roberts et al. (38) all reported that periodic ophthalmia developed in horses following inoculation with leptospires. The lesions required several months to a year for development. 

Morter et al. (46) reported that ocular lesions developed in 10 *L. pomona* inoculated ponies within 15 months post-exposure while none of the nine control ponies developed lesions. Ocular lesions began with serous lacrimation independent of conjunctivitis and keratitis followed by a remission period. Subsequent lesions were more severe and consisted of corneal vascularization, hypopyon, hyphema, photophobia, miosis, purulent lacrimal discharge and keratitic precipitates in the anterior chamber.

Although most efforts to recover leptospires from ocular fluids have been unsuccessful, some isolates have been reported (Hartwig et al. (31), Yager (50) and Kathe as reported by Gsell (51).

The studies described here were designed to determine leptospiral agglutination rates in selected equine populations.

**Materials and Methods**

The three equine populations were selected as follows:

**GROUP I.** Sera were collected either from horses admitted as suspect leptospirosis cases to the University of Illinois College of Veterinary Medicine, Large Animal Clinic, or submitted to the Illinois Department of Agriculture Diagnostic Laboratory at Urbana by practicing veterinarians for leptospiral agglutination tests. All sera were tested using the microscopic agglutination (MA) technique against *L. pomona* antigen. In addition, sera collected after 1958 were tested using the MA technique against the following antigens: *L. autumnalis*, *L. canicola*, *L. grippotyphosa*, *L. hardjo*, and *L. icterohemorrhagiae*.

**GROUP II.** Sera were collected from horses admitted to the University of Illinois Veterinary College Large Animal Clinic in 1968 without prior knowledge of admitting diagnosis. Also, a smaller number of sera were collected from horses at the University of Illinois Research Farm in Urbana and from privately owned horses. These sera were tested using the MA technique against the six antigens listed above.

**GROUP III.** Blood samples were collected from thoroughbreds during the 1965 season (May-July) in connection with an equine influenza study at a southern Illinois track. The horses were selected on the basis of trainer cooperation rather than a predetermined sampling technique. Initial blood samples were obtained between May 6 and June 3, and second samples were obtained between July 7 and July 22. The age, sex, barn and stall location was obtained from the trainer at the time of the first blood collection. The age and sex distribution of the total horse population at the track was obtained from the Track Steward's official records. Blood samples were collected in 20 ml. sterile Vacutainers and stored at refrigerator temperature for
approximately four days before the clots were separated from the sera. Sera were tested using the MA technique against the six antigens listed above.

Results
Diagnostic sera from Group I animals had decreasing reactor rates against *L. pomona* when grouped into 4 year periods from 1953 through 1968 (Figure 1). This reduction paralleled rates in Illinois cattle and swine sera. Sera from 273 of these horses were also tested against five other leptospiral antigens (Table 4). Sixteen sera had MA titers of 1%100 or greater against *L. canicola*. Six sera reacted against *L. autumnalis* antigen. Four of these sera were submitted in 1967, one each in 1964 and 1968. Two sera collected in 1961 reacted against *L. grippotyphosa* while one in 1964 reacted against *L. hardjo* antigen.

Sera from 19 of the 163 horses in Group II had antibody at the 1:100 dilution or greater. Seven sera reacted against *L. autumnalis*, 4 against *L. grippotyphosa* and 1 against *L. hardjo*. Six sera contained antibodies against 2 serotypes. Four of these had *L. autumnalis* antibody in combination with *L. pomona* (2), *L. canicola* (1), and *L. icterohemorrhagiae* (1). Two reacted against *L. icterohemorrhagiae* in combination with *L. canicola* (1) and *L. grippotyphosa* (1). The other serum reacted against three serotypes, *L. autumnalis*, *L. canicola* and *L. grippotyphosa*.

The Group III samples represented 101 of the 1164 horses on the race track. The age and sex of this sample population was similar to that of the track population except that only 5.6% of the animals 10 years of age or older were tested in contrast to 8.7% of the other age groups. Eight of the 11 barns on the track were represented in the sample population. Seven sera collected during the first bleeding were reactive at the 1:100 dilution, 6 against *L. canicola* antigen and 1 against *L. icterohemorrhagiae* antigen. None of the sera collected during the second bleeding period reacted at the dilution of 1:100 or greater. There were no significant differences when the data were examined according to age, sex or stall location.

Discussion
The use of the *L. pomona* bacterin to prevent infections in Illinois cattle and swine became widespread in the mid-1950's. The decrease in reaction rates in equine sera at that time perhaps indicates that cattle and/or swine were serving as an important source of infection for horses. Since data are not available on the extent of *L. pomona* bacterin use in Illinois horses, it is possible that part of the decrease in equine reactor rates was due to bacterin use in this species. Although unlikely, it is also possible that some unexplained factor or set of factors, was responsible for the decrease in all three species.

The comparatively low *L. pomona* reactor rate (1.2%) in sera from Group II animals is probably closer to the true reactor rate in Illinois horses than in the Group I rate since Group II animals were not suspect leptospirosis cases. It is interesting that all 19 (12.4%) reactive sera from Group II contained antibody to serotypes other than *L. pomona* while 25 (9.2%) of 273 diagnostic sera collected from Group I animals between 1959 and 1968 reacted to these serotypes. Second sera from Group III (race track) did not react at dilutions greater than incomplete
1:100. However, seven animals did react at this dilution against *L. ballum*. Initial sera from these seven animals had been completely non-reactive at the 1:100 dilution. The significance of these titers is not known. *L. pomona* and *L. canicola* infections appear more prevalent in horses.
FIGURE 1
REACTOR RATES OF *L. Pomonap* IN ILLINOIS HORSES, CATTLE AND SWINE, 1953-68

---

HORSES
CATTLE
SWINE

PERCENT POSITIVE

0 1953-56 1957-60 1961-64 1964-68

MICROSCOPIC AGGLUTINATION TITER OF 1:100 OR GREATER
<table>
<thead>
<tr>
<th>GEOGRAPHIC AREA</th>
<th>INVESTIGATOR</th>
<th>SERA TESTED</th>
<th>PERCENT POSITIVE</th>
<th>SEROTYPES INVOLVED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia, Africa, Far East</td>
<td>Wellington (1)</td>
<td>92</td>
<td>46.7%</td>
<td>icterohemorrhagiae, mitis, pomona</td>
</tr>
<tr>
<td>Australia</td>
<td>Shirahata (2)</td>
<td>96</td>
<td>2.1%</td>
<td>NG</td>
</tr>
<tr>
<td>Madagascar</td>
<td>Koloche-Erber (3)</td>
<td>30</td>
<td>16.7%</td>
<td>ballum, canicola, icterohemorrhagiae, sejroe, sentoti</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bohemia &amp; Moravia</td>
<td>Sebek (4)</td>
<td>130</td>
<td>NG</td>
<td>bratislava, grippotyphosa, icterohemorrhagiae</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>Asmera (5)</td>
<td>70</td>
<td>28.6%</td>
<td>grippotyphosa</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>Sova (6)</td>
<td>891</td>
<td>10.7%</td>
<td>sejroe</td>
</tr>
<tr>
<td>England</td>
<td>Alston &amp; Broom (7)</td>
<td>108</td>
<td>NG</td>
<td>canicola, icterohemorrhagiae</td>
</tr>
<tr>
<td>Germany</td>
<td>Mochmann (8)</td>
<td>1182</td>
<td>31.3%</td>
<td>australis, canicola, grippotyphosa, hebdomadis, icterohemorrhagiae, pomona, sejroe</td>
</tr>
<tr>
<td>Germany</td>
<td>Kathe (9)</td>
<td>162</td>
<td>37.7%</td>
<td>grippotyphosa, australis, icterohemorrhagiae</td>
</tr>
<tr>
<td>Hungary</td>
<td>Fuzi (10)</td>
<td>55</td>
<td>69.1%</td>
<td>ballum, canicola, grippotyphosa, icterohemorrhagiae, pomona, sejroe</td>
</tr>
<tr>
<td>Hungary</td>
<td>Kiszel (11)</td>
<td>38</td>
<td>65.8%</td>
<td>pomona, sejroe, ballum, hyos, icterohemorrhagiae</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Grant (12)</td>
<td>71</td>
<td>33.8%</td>
<td>icterohemorrhagiae, kremastos</td>
</tr>
<tr>
<td>Morocco</td>
<td>Blanc (13)</td>
<td>13</td>
<td>15.4%</td>
<td>canicola</td>
</tr>
<tr>
<td>Poland</td>
<td>Parnas (14)</td>
<td>181</td>
<td>40.3%</td>
<td>NG</td>
</tr>
<tr>
<td>Portugal</td>
<td>Fraga de Azevendo (15)</td>
<td>163</td>
<td>4.9%</td>
<td>Ballum, hyos</td>
</tr>
<tr>
<td>Rumania</td>
<td>Combiesco (16)</td>
<td>371</td>
<td>60.9%</td>
<td>canicola, grippotyphosa, icterohemorrhagiae, mitis, pomona, sejroe</td>
</tr>
<tr>
<td>Serbia</td>
<td>Trbic (17)</td>
<td>20</td>
<td>NG</td>
<td>icterohemorrhagiae, pomona</td>
</tr>
<tr>
<td>Spain</td>
<td>Covalida (18)</td>
<td>NG</td>
<td>NG</td>
<td>icterohemorrhagiae, grippotyphosa, pomona</td>
</tr>
<tr>
<td>Sweden</td>
<td>Wendt (19)</td>
<td>1000</td>
<td>12.0%</td>
<td>canicola, grippotyphosa, icterohemorrhagiae, pomona</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Heussen (20)</td>
<td>554</td>
<td>43.3%</td>
<td>australis, grippotyphosa, icterohemorrhagiae, pomona, sejroe</td>
</tr>
<tr>
<td>USSR</td>
<td>Konrad (21)</td>
<td>626</td>
<td>40.4%</td>
<td>australis, ballum, canicola, grippotyphosa, icterohemorrhagiae, pomona, sejroe</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>Zaharija (22)</td>
<td>22</td>
<td>72.7%</td>
<td>pomona</td>
</tr>
<tr>
<td>Denmark</td>
<td>Fennestad (7)</td>
<td>NG</td>
<td>NG</td>
<td>canicola, grippotyphosa, icterohemorrhagiae, saxkalbing, sejroe</td>
</tr>
<tr>
<td>Israel</td>
<td>vander Holden (14)</td>
<td>17</td>
<td>58.8%</td>
<td>grippotyphosa</td>
</tr>
<tr>
<td>Israel</td>
<td>vander Holden (15)</td>
<td>36</td>
<td>19.4%</td>
<td>canicola</td>
</tr>
<tr>
<td>North, South America</td>
<td>Savino (23)</td>
<td>115</td>
<td>NG</td>
<td>hyos, pomona</td>
</tr>
<tr>
<td>Argentina</td>
<td>Varela (24)</td>
<td>15</td>
<td>26.7%</td>
<td>icterohemorrhagiae</td>
</tr>
<tr>
<td>Mexico</td>
<td>Grant (16)</td>
<td>71</td>
<td>33.8%</td>
<td>icterohemorrhagiae, kremastos</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Bryans (25)</td>
<td>1027</td>
<td>NG</td>
<td>canicola, icterohemorrhagiae, pomona</td>
</tr>
<tr>
<td>USA</td>
<td>Platt (26)</td>
<td>2</td>
<td>100.0%</td>
<td>icterohemorrhagiae</td>
</tr>
</tbody>
</table>

*as defined by investigator  NG – Not Given
<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>COUNTRY</th>
<th>INVESTIGATOR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. canicola</em></td>
<td>Germany</td>
<td>Hartwigk (27)</td>
</tr>
<tr>
<td></td>
<td>USSR</td>
<td>Karimova (28)</td>
</tr>
<tr>
<td><em>L. grippotyphosa</em></td>
<td>Poland</td>
<td>Zwierz (29)</td>
</tr>
<tr>
<td><em>L. icterohemorrhagiae</em></td>
<td>France</td>
<td>Rossi (30)</td>
</tr>
<tr>
<td><em>L. pomona</em></td>
<td>Hungary</td>
<td>Fuzi (31)</td>
</tr>
<tr>
<td></td>
<td>Hungary</td>
<td>Hirt (32)</td>
</tr>
<tr>
<td></td>
<td>USSR</td>
<td>Ananyin (33)</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Roberts (34)</td>
</tr>
<tr>
<td></td>
<td>Yugoslavia</td>
<td>Brudnjak (35)</td>
</tr>
<tr>
<td></td>
<td>Yugoslavia</td>
<td>Zahrenia (36)</td>
</tr>
<tr>
<td></td>
<td>Yugoslavia</td>
<td>Zahrenia (37)</td>
</tr>
<tr>
<td><em>L. saxkoebing</em></td>
<td>Hungary</td>
<td>Fuzi (31)</td>
</tr>
<tr>
<td><em>L. sejroe</em></td>
<td>Hungary</td>
<td>Hirt (32)</td>
</tr>
<tr>
<td></td>
<td>Hungary</td>
<td>Kemenes (38)</td>
</tr>
<tr>
<td></td>
<td>Yugoslavia</td>
<td>Zahrenia (37)</td>
</tr>
</tbody>
</table>
### Table 3.

**ASSOCIATION BETWEEN LEPTOSPIRAL ANTIBODIES AND PERIODIC OPHTHALMIA IN HORSES**

<table>
<thead>
<tr>
<th>INVESTIGATOR</th>
<th>POPULATION DESCRIPTION</th>
<th>NUMBER TESTED</th>
<th>PERCENT POSITIVE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryans (25)</td>
<td>Periodic Ophthalmia</td>
<td>23</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>Brood Mares</td>
<td>512</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Weanling Horses</td>
<td>492</td>
<td>0%</td>
</tr>
<tr>
<td>Heusser (20)</td>
<td>Periodic Ophthalmia</td>
<td>263</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>291</td>
<td>12%</td>
</tr>
<tr>
<td>Mochmann (8)</td>
<td>Periodic Ophthalmia</td>
<td>64</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>No Periodic Ophthalmia</td>
<td>1118</td>
<td>27%</td>
</tr>
<tr>
<td>Woods &amp; Davis (44)</td>
<td>Periodic Ophthalmia</td>
<td>12</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>30</td>
<td>13%</td>
</tr>
<tr>
<td>Yager (45)</td>
<td>Periodic Ophthalmia</td>
<td>35</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>86</td>
<td>12%</td>
</tr>
</tbody>
</table>

*as defined by investigator
### TABLE 4

**Reactor Rates of Equine Sera to Six Leptospiral Serotypes 1953-1968**

<table>
<thead>
<tr>
<th>Population</th>
<th>Sera Tested</th>
<th>L. Pomona</th>
<th>L. Autumnalis</th>
<th>L. Ballum</th>
<th>L. Canicola</th>
<th>L. Grippotyphosa</th>
<th>L. Hardjo</th>
<th>L. Icterohemorrhagiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I (Diagnostic Sera)</td>
<td>273</td>
<td>19.2%**</td>
<td>2.2%</td>
<td>NT</td>
<td>5.9%</td>
<td>0.7%</td>
<td>0.4%</td>
<td>0</td>
</tr>
<tr>
<td>GROUP II (Clinic-Farm)</td>
<td>163</td>
<td>1.2%</td>
<td>7.3%</td>
<td>NT</td>
<td>1.8%</td>
<td>3.6%</td>
<td>0.6%</td>
<td>1.8%</td>
</tr>
<tr>
<td>GROUP III (Racetrack)</td>
<td>101</td>
<td>0</td>
<td>NT</td>
<td>0</td>
<td>5.9%</td>
<td>0</td>
<td>0</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

* Microscopic agglutination titer of 1:100 or greater
** Total of 807 sera tested against L. Pomona in GROUP I
NT = Not tested
REFERENCES

EQUINE LEPTOSPIROSIS


Summary

Serologic surveys indicate leptospiral infections occur frequently in horses and can be caused by a variety of serotypes. The most commonly recognized form of the disease in horses is periodic ophthalmia, although acute illnesses have been reported. Diagnostic sera collected from horses had decreasing reactor rates against *L. pomona* 1953-68. This reduction paralleled rates in Illinois cattle and swine. Twenty-five of 273 sera from these horses contained antibodies against *L. autumnalis, L. canicola, L. grippotyphosa, L. hardjo*, or *L. icterohemorrhagiae*. Nineteen of 163 sera from horses without signs of leptospiral infection had microscopic agglutination titers of 1:100 or greater against one of the 6 serotypes. Sera from 101 horses at an Illinois race track had *L. canicola* or *L. icterohemorrhagiae* antibodies (6.9%).
CHRONIC LEPTOSPIRA HARDJO
AND LEPTOSPIRA HEBDOMADIS INFECTION
IN A FIFTY COW HERD OF DAIRY CATTLE,
A CASE HISTORY

by
Larry L. Smith, D.V.M.
Lodi, Wisconsin

The following is a case history of chronic Leptospira hardjo and Leptospira hebdomadis infection in a fifty cow herd of dairy cattle owned by a client in our practice in south central Wisconsin. The herd was maintained at 50 cows by purchasing replacement animals when necessary.

Between April, 1965, and April, 1967, 38 cows aborted. The first cow aborted on April 16, 1965, with no symptoms other than abortion. The serologic status of the cow for brucellosis or leptospirosis was not determined. The next cow aborted during the last part of April, 1965. A neighboring veterinarian examined the cow, diagnosed leptospirosis, and vaccinated the herd with L. pomona vaccine.* All new cows purchased after this date were vaccinated with L. pomona vaccine** and the entire herd revaccinated at 6 months, then 1 year later.

Due to the owner's inconsistent request for veterinary service, the first serum sample was not submitted until after an abortion (cow 17) in September of 1965. (All laboratory tests were performed at the Central Animal Health Laboratory, Madison, Wisconsin). This sample was negative for all 6 serotypes tested (L. pomona, L. icterohemorrhagiae, L. Hardjo, L. autumnalis, L. hebdomadis, and L. grippotyphosa).

The next two serum samples were submitted in January of 1966. Cow 57 was negative for all serotypes, while a white heifer was positive at a 1/1000 titer for L. hebdomadis. At this time it was felt that some cross immunity developed to other serotypes of leptospirae from the L. pomona vaccination, but subsequent observations make this seem untrue. Thus in all the abortions from January 18, 1966, through June 28, 1966, some other cause was sought. The feed, roughages, and concentrates, were analyzed for nitrates. In all cases there were not enough nitrates present to be detected by chemical means. The first water sample analyzed for nitrates in early March 1966 contained 38 ppm of nitrates.

On March 28, 1966, Dr. Eveleth of the Central Animal Health Laboratory, Madison, Wisconsin, accompanied me to the farm. The following diagnostic tests were run:

1. Blood samples were taken from 5 cows that had previously aborted. Two cows were negative for all 6 serotypes, 2 cows were positive for L. pomona at a 1/1000 dilution, and cow 17 was positive for L. hardjo and L. hebdomadis at a 1/100 dilution. When checked 9 months earlier, this cow was negative.

2. A water sample analyzed at this time showed an increase in nitrates from

* Norden, Omaha, Nebraska
** Fort Dodge Laboratories, Fort Dodge, Iowa

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38 ppm to 50 ppm.
3. Vaginal swabs taken from cows passing exudate yielded no significant pathogens.

At this time the significance of the leptospirae serotypes was not understood, but the 50 ppm of nitrates in the water was considered a cause of abortions.

The cows continued to abort. Three fresh fetuses were taken to the laboratory during the last of June and the first week in July of 1966. They were cultured for PPLO, vibrio, bacteria, and checked on dark field for leptospirae. The only potential pathogen isolated was a hemolytic streptococcus. Vaginal swabs from the cows that aborted were cultured and a coliform was isolated. On July 27, 1966, serum samples from 4 cows that had aborted during June and July, 1966 and cow 17 (aborted September 1965) were submitted. The titer of a cow that had aborted 38 days earlier was now 1:100 for L. hardjo and continued at a 1/100 for L. pomona. The L. pomona titer probably was due to the vaccination 3 months earlier. The titer of cow 17 had increased to 1/100 for L. hardjo and 1/100 for L. hebdomadis. Two other cows had serum titers of 1/1000 for L. hardjo and 1/100 for L. hebdomadis. The conversion of these animals with overt signs of disease to a positive serologic status was considered confirmatory of a diagnosis of leptospirosis. The leptospirosis was thought to be the result of infection with L. hardjo or L. hebdomadis. Vaccines were not available.

The abortions continued. On April 24, 1967, another fresh fetus from cow 58 was submitted to the Central Animal Health Laboratory and cultured for vibrio, brucella and fungus, and examined under the dark field for leptospirae. The above tests were negative. About 3 days later cow 58 began passing bloody urine. At this time blood samples for leptospirae serologic evaluation were taken from the only 10 cows that had aborted and were still in the herd. Other cows that had aborted were felt to be non-profitable and sold for salvage. Sera of six of the cows were positive for L. hardjo at 1/1000 dilution, 2 cows, 1/100, for L. hardjo, and 5 of these same cows had detectable serum agglutinins for L. hebdomadis at a 1:100 dilution.

With the lack of success of finding or culturing the leptospirae from the fresh aborted fetuses cultural isolation from the ujing of the aborted cows was undertaken. Midstream ujing samples were collected in sterile containers from the last 8 cows that aborted. These were submitted for dark field examination and culture. Leptospirae were seen on dark field examination in the urine of the cow passing bloody urine. Isolation attempts were unsuccessful. Rather than repeat the attempts to culture the urine, Dr. Ott at Fromm Laboratories was contacted. Through Dr. Ott's cooperation, an experimental bivalent vaccine was made available. There have been no abortions since the initial vaccination in January, 1968. Vaccination was repeated in 6 months, and yearly since.

An interesting fact is that from January, 1966, to vaccination in January, 1968, 32 cows were treated for uterine infection after abortions or calving. After vaccination, this was reduced significantly, probably no more than 6. The DHIA herd average for butter fat was 535 pounds in 1964, 515 pounds in 1965, 448 pounds in 1966, 442 pounds in 1967, and 422 pounds in 1968. To September of this year, the average had risen to 460 pounds of butter fat. Cow 58 with the leptospiruria produced 706 pounds of butter fat the year before abortion. During
lactation, after abortion, she dropped to below 200 pounds of butter fat. During 1966, twenty-six cows were sold and replacements purchased because of abortion and in 1967, thirteen cows were sold because of abortion. Thus in 2 years, the loss of production, replacement costs for 38 cows, and the cost of veterinary services represented a great economic loss.

CONCLUSIONS:
1. Leptospirosis caused by serotypes other than L. *pomona* are extremely difficult for the practicing veterinarian to diagnose. Frequently the cow is sold soon after abortion and important paired serum samples are not available.
2. Few of the cows that aborted showed signs other than abortion, transient anorexia and decreased milk production. Only one cow out of the 38 that aborted passed bloody urine.
3. In our practice, 50 ppm of nitrates in the water will not cause abortion. We have farms with a higher water nitrate level, but abortion is not a problem on these farms.
4. The experimental bivalent vaccine (*L. hardjo* and *L. hebdomadis*) has given excellent results in this herd.

ACKNOWLEDGEMENTS:
I want to thank Dr. Lyle and his staff at the Central Animal Health Laboratory for their continual interest and assistance without which this diagnosis could not have been made.

I want to thank Dr. Ott of Fromm Laboratories for making their experimental bivalent vaccine available.
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

Chairman: R. L. Morter, West Lafayette, Ind.

The Committee reviewed the previous year's report and information that has become available during the current year. The Committee commends the Veterinary Biologics Division of the Agricultural Research Service for Biological Products Memo No. 44 which is in substantial agreement with the previous recommendations of the Committee.

In as much as multivalent vaccines have been recommended for use in areas where several serotypes are prevalent, the Committee urges that the state diagnostic laboratories or other designated agencies in all states undertake surveys to establish the prevalence of the various serotypes in their animal populations. For such surveys to provide meaningful information, acceptable serological methods must be employed. The Diagnostic Services at the National Animal Disease Laboratory is encouraged to provide training in leptospiral serological methods. This training would help establish uniformity in the application of serologic procedures and make such surveys more meaningful.

It is further recommended that the Biologics Division of the Agricultural Research Service evaluate and license all leptospiral antigens intended for use in the macroscopic agglutination test for diagnosing leptospirosis in animals. The Committee further recommends that licensing of antigens be limited to those serotypes that have been demonstrated prevalent in livestock and companion animals (Leptospira pomona, L. canicola, L. icterohemorrhagiae, L. grippotyphosa, and L. hardjo).

The results of studies with chemotherapeutic agents indicate that the carrier state can be eliminated in cattle and swine by parenteral administration of 25 mg/kg of dihydrostreptomycin. In conjunction with bacteriologic and serologic tests, the use of dihydrostreptomycin may permit the introduction and movement of valuable breeding stock which may have low, persistent serologic reactions.

The Committee wishes to reiterate the opinion expressed in previous reports that leptospirosis is not amendable to eradication because of the numerous serotypes encountered, the difficulty of detecting carrier animals and the wide range of domestic and wild animal hosts. Therefore, the Committee emphasized that adequate financial support is necessary for the development of improved diagnostic procedures and control measures. Specific emphasis should be placed on the development of effective immunizing agents. We respectfully submit this report to the Executive Committee for approval and suggest that the work of this committee be continued.
BOVINE EPHEMERAL FEVER
II. Responses of Cattle to Attenuated and Virulent Virus

W. P. Heuschele, D.V.M., Ph.D.*
and
D. C. Johnson, D.V.M.**

INTRODUCTION

Bovine ephemeral fever (EF), or "three-day sickness," is a noncontagious arthropod-transmitted virus disease of cattle. It is characterized by sudden fever, stiffness, lameness, serous nasal and ocular discharge, drooling, and rapid recovery. In milk cows, there is complete cessation of lactation during the acute illness and milk production is greatly reduced until the next lactation. First described in 1867 in South Africa, the disease has since been identified in many parts of tropical Africa, the Middle East, India, Pakistan, Indonesia, Japan, and Australia.1

Mackerras et al.9 reported the clinical and pathologic reactions of cattle to experimental infection with virulent ephemeral fever virus (EFV) and some characteristics of the etiologic agent. Clinical signs above were accompanied by neutrophilia and, frequently, leukocytosis. The infection could not be transmitted to horses, sheep, goats, dogs, rabbits, guinea pigs, rats, or mice. More recently, van der Westhuizen14 reported on the adaptation of EFV to suckling mice by intracerebral inoculation, and some responses of cattle to inoculation with mouse-passaged EFV. After 8-10 passages in suckling mouse brain EFV became adapted and inoculation of mice resulted in 100% mortality after 2 and 3 days. Cattle inoculated with a single dose of 3rd and 9th mouse passed EFV, either subcutaneously or intravenously (I.V.), failed to develop clinical reactions or detectable neutralizing antibody and were susceptible to virulent EFV inoculated 4 weeks later. Cattle given 3 subcutaneous inoculations of 9th mouse passage EFV with oil adjuvant at weekly intervals developed neutralizing antibodies and were resistant to virulent EFV 14 days after the last inoculation.

Australian workers3 had similar experience with EFV adapted to suckling mouse brain. After 3 to 6 passages, the virus was no longer virulent for cattle and required multiple inoculations to elicit detectable neutralizing antibody or resistance to immunity challenge with virulent EFV. Both van der Westhuizen14 and the Australian workers conduct virus neutralization tests in suckling mice inoculated intracerebrally. In addition, the Australian workers have adapted EFV to baby hamster kidney (BHK) cell line cultures in which cytopathic effect (CPE) is evident 2 to 3 days postinoculation (DPI) and in which virus neutralization tests proved to be as satisfactory as those in mice.3

This report describes clinical, hematologic, and serologic responses of cattle inoculated with virulent EFV and EFV attenuated by passage in suckling mouse

* Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, N.Y. 11944.

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brains and BHK cell cultures.

MATERIALS AND METHODS

Viruses
Virulent EFV was obtained from Dr. A. E. Pierce, Chief, Division of Animal Health, Animal Health Research Laboratory, Parkville, Victoria, Australia, as crude buffy coat suspension from infected bovine blood. Additional virulent EFV was produced by inoculating 2 ml. of the above material I.V. into one steer. At the peak of clinical response (4 and 5 DPI), blood was obtained in heparin (100 unit/ml.) and the buffy coat fraction obtained by centrifugation and suspended (10%) in phosphate buffered saline, (PBS), pH 7.4, 0.1 M, was stored at -350° until used.

Mouse-adapted EFV was obtained from Dr. R. L. Doherty, Director, The Queensland Institute of Medical Research, Brisbane, Australia, as 6th mouse brain passage suspension in 7.5% bovine albumin in borate buffer, pH 9.0. Several additional passages in suckling mouse brains were made and the 8th mouse-brain-passage of EFV was adapted to BHK cells and subsequently to monkey kidney cell lines (MS and Vero) as previously described.4

Animals
Adult grade Hereford and Hereford-Shorthorn-cross steers were used. Clinical and hematologic examinations (erythrocyte and leukocyte enumeration, differential leukocyte enumeration, and hematocrit) were conducted at various intervals.

Serology
Preinoculation sera and sera obtained from steers at various intervals after inoculation were tested for antibody by the agar gel diffusion precipitin (AGDP) plaque neutralization (PN), and virus neutralization (VN) tests.

Antigens for AGDP test were as follows:
(1) 20% suspension of EFV-infected mouse brain passage 10 in PBS (pH 7.4, 0.1 M), harvested when mice were in extremis.14
(2) EFV – 12th and 13th passage in BHK cells, cell debris concentrated 250 X and 100 X, respectively, by centrifugation at 1500 G for 30 minutes.
(3) EFV – 14th passage in MS cells, cell debris concentrated 90 X by centrifugation.
(4) Buffy coat fraction of blood collected from steers 33, 411, and 413 on the first and second days of acute EF illness, suspended 1:5 in PBS.

For the test 1% Ionagar No. 2 (Difco Laboratories, Detroit, Mich. 48201) in 0.85% saline or PBS was poured into petri dishes to a depth of approximately 3 mm, or on glass microslides to a thickness of 2 mm. Wells were cut in a rosette pattern and were filled with appropriate reagents.

VN tests were conducted with EFV adapted to MS cells (10th-12th passage) in roller tube MS cell cultures. Constant serum at a final dilution of 1:10 was mixed with decimal dilutions of EFV, incubated at 37°C for 60 minutes and 0.1 ml. was inoculated per tube. Maintenance medium was added after a 45-minute absorption period. Median CPE titers were computed by the method of Karber8 and
neutralization indices (N.I.) were obtained by subtraction of the log10 titer of serum/virus assays from the log10 titer of virus with 10% normal bovine serum. Figure 1 illustrates the character of CPE obtained in MS cells 48-72 hours postinoculation with adapted EFV.

PN tests were conducted with mixtures of serum at various final dilutions and 30-100 PFU EFV-MS 11 (11th passage in MS cells) incubated 60 minutes at 37°C, and inoculated onto Vero cell monolayers in 4-oz. prescription bottles in 0.5-ml. amounts. Overlay media and other aspects of the test were as previously described for plaque assay of EFV. Typical plaques formed with EFV are shown in Figure 2. Plaque counts were made between 7 and 10 DPI.

EXPERIMENT AND RESULTS

Inoculation of cattle with virulent EFV

Three of four cattle inoculated I.V. with virulent EFV developed fever after 4 DPI; one was febrile 3 DPI (Table 1). Fever persisted for an additional day, but body temperature returned to normal by the third day after onset of illness. Fever was accompanied by drooling, anorexia, serous ocular discharge, serous or sero-mucoid nasal discharge, stiffness, depression, weakness, and lameness. Nasal and conjunctival mucous membranes were injected. There were no apparent gross lesions in the mouth or on the feet. Hyperpnea, probably associated with the fever, was also evident. These clinical signs were present for 2 days and by the 3rd day only a slight weakness in the hindquarters was evident.

The only hematologic alteration observed was a rise in the percentage of neutrophils in differential leukocyte counts to a mean of 63% (normal range: 10-40%) on the first day of fever. By the 5th day after onset of illness, the neutrophil value was within normal limits. Total leukocyte counts, erythrocyte counts, and hematocrit values did not change appreciably.

One steer (43) was killed 4 DPI, the day after the appearance of clinical signs of EF. Apparent gross lesions were moderate edema of lymph nodes, congestion of ocular and nasal mucous membranes, and some increase in synovial fluid with fibrinous flocculation in carpal, tarsal, humeroscapular, and acetabular joints. There was also slight congestion in joint capsules. On histologic examination, there was moderate dilation of lymph node sinuses with edema fluid in most lymph nodes examined. Abundant iron-pigment was present in medullary macrophages. The spleen also contained an abundance of pigment-laden round cells. Joint capsules and synovia had small focal areas of neutrophilic and histiocytic infiltration and moderate hyperemia. There was a small amount of capillary hemorrhage in one section of joint capsule. There was extensive diffuse lymphoid and histiocytic cell infiltration of the subepithelial connective tissue of the conjunctiva. In some areas, these round cell infiltrations were concentrated around capillaries and extended into accessory glands (apocrine and sebaceous). The overlying stratified epithelium also contained some infiltrated round cells and numerous eosinophils in some areas. The nasal mucosa was hyperemic and had scattered foci of round cell and eosinophil infiltration. There was engorgement of alveolar capillaries but no other lesion in lung sections. No lesions of significance were seen in any other tissues.
Inoculation of cattle with attenuated EFV

Two steers (301 and 281) were inoculated subcutaneously at weekly intervals for 4 weeks with 5 ml., 8th mouse brain passage EFV (5 x 10^8 MDL_50) with an equal volume of Freund's incomplete adjuvant (Difco Laboratories, Detroit, Mich. 48201). No clinical signs were observed during this inoculation course. There was a slight rise in neutrophil counts 1 day following each inoculation. However, a steer similarly inoculated with normal mouse brain suspension and adjuvant had similar increases in neutrophils 1 DPI.

One steer (299) was inoculated subcutaneously at weekly intervals for 5 weeks with 5 ml. of 10th BHK cell passage EFV culture fluid (5 x 10^5.0 TCID_50) with an equal volume of Freund's incomplete adjuvant. Another steer was similarly inoculated with normal BHK cell culture fluid. No clinical abnormalities were noted in either steer until the third inoculation when steer 299 (inoculated with BHK passaged EFV) had a fever (104°F) the following day. No other abnormal signs were observed and the next day the body temperature returned to normal (101.6°F). The neutrophil count of steer 299 showed marked increase 1 day after each inoculation of attenuated EFV. No similar change in neutrophil count was observed in the steer inoculated with normal BHK culture fluid.

Postinoculation sera obtained from steer 299 (inoculated with BHK passaged EFV), 14 days after the last inoculation, had the highest titers of VN and PN antibodies and produced precipitin bands with all EFV antigen preparations tested (Figure 3, Table 2). Precipitin reactions did not occur between steer 299 immune serum and uninfected cell culture, mouse brain or buffy coat antigen preparations. Precipitating antibodies were first detected in serum obtained 7 days following the second inoculation of BHK-adapted EFV (14 days after the first does). The 1:10 dilution of steer 299 serum collected 14 days following the last inoculation of EFV had a N.I. of -- 4.75. The median PN titer of this serum was 1:3840.

Sera obtained 14 DPI from steers 33, 411, and 413 (inoculated with virulent EFV) had N.I. of 2.0, 2.0, and 1.0, respectively (Table 2). These sera produced precipitin reactions with the 4 antigens irregularly and when present, bands were very faint but had identity with bands produced by steer 299's sera, and with an EF convalescent serum (No. S6849) obtained from a bovine animal experimentally infected in Australia (furnished by Dr. A. E. Pierce, Chief, Division of Animal Health, Animal Health Research Laboratory, Parkville, Victoria, Australia). This serum had a PN titer similar to that of convalescent sera from the above cattle in our study (Table 2).

The cattle inoculated with EFV passaged in mouse brain failed to produce any detectable precipitating or neutralizing antibody (Table 2).

The immunity of steers 33 and 299 was challenged by intravenous inoculation with virulent EFV 23 days after the original inoculation with virulent EFV, and 66 days following the last inoculation with BHK cell passaged EFV, respectively. Neither steer had any clinical or hematologic response following this challenge inoculation, indicating immunity.

Serums collected from these steers, 7 and 14 days after challenge inoculation, had no change in antibody levels.

Steers 301 and 281 were killed before virulent EFV became available, and hence their immune state could not be assessed by challenge inoculation.
Although EF does not exist in the United States, arthropods and cattle from endemic areas represent potential sources for the introduction of this disease. The ceratopogonid gnats of the genera *Ceratopogon* and *Culicoides*, which have been suspect as the principal vectors of EFV in endemic areas, are found in the United States.

The diagnosis of EF has been based on: 1) clinical features, 2) isolation and transmission of the virus by IV inoculation of buffy coat fractions of blood from sick animals to susceptible cattle, and 3) demonstration of virus neutralizing and complement fixing antibodies in convalescent sera. The pathologic lesions observed in one steer in our study were similar to those previously described for EF. No pathognomonic lesions for EF were observed.

The development of plaque assay and neutralization methods herein described, and the AGDP test for EFV add additional procedures for the study and diagnosis of EF. Buffy coat fractions from cattle with clinical EF should be tested against high titer antiserum in the AGDP test. Plaque neutralization affords a more sensitive test for EF antibody than AGDP or VN. Van der Westhuizen and Australian workers have successfully immunized cattle using multiple inoculations of suckling mouse-brain-adapted EFV with adjuvant. Our studies suggest that EFV passaged in cell cultures (BHK) subsequent to mouse adaptation, may be equally efficacious for immunizing cattle against EF. The lack of response to mouse-adapted EFV in our studies may have been due to a loss of infectivity of the preparations of virus upon storage.

Two diseases of cattle with clinical features similar to EF occur in Japan. One, bovine epizootic fever (BEF) is virtually identical with respect to epizootiologic and clinical features. The virus involved appears similar physicochemically to vesicular stomatitis virus, *i.e.*, it has a bullet shape, size 80 x 140 μm, and is inactivated by diethyl ether and deoxycholate. A recent serologic comparison of the virus of BEF and EFV indicated some antigenic relationship between the two agents. Further studies on the relationship and characteristics of these agents are currently being done at the Plum Island Animal Disease Laboratory.

The second disease, caused by the so-called Ibaraki virus, has been confused with BEF. Clinically this disease resembles bluetongue infections in cattle in the United States. Studies are underway to determine the relationship of the disease with respect to bluetongue, EF and BEF.

**SUMMARY**

The clinical and immunologic responses of cattle to inoculation with virulent and attenuated ephemeral fever virus are described. The development of a plaque neutralization and precipitin test are reported and provide additional methods for the diagnosis and study of ephemeral fever. The plaque neutralization test appeared to be the most sensitive method for the detection of EF antibody. EFV passaged in BHK cells was satisfactory for immunization of cattle when multiple inoculations were given with adjuvant.
Fig. 2. Plaques formed by EFV in Vero cell monolayers under agar, 10 DPI.
A = EFV antiserum #299

1 = EFV BHK 12 (250X)

2 = EFV BHK 13 (100X)

3 = EFV MB 11 (20%)

4 = EFV MS 14 (90X)

Fig. 3 Precipitin bands formed between EFV antigens and EF hyperimmune serum.
A. Antiserum obtained 14 days after last inoculation with 10th BHK passage of EFV.
1. 12th BHK passage of EFV, cell debris concentrated 250 X.
2. 13th BHK passage of EFV, cell debris concentrated 100 X.
3. 11th mouse brain passage of EFV, 20% suspension in PBS with 10% bovine serum.
4. 14th MS passage of EFV, cell debris concentrated 90 X.
Table 1. CLINICAL SIGNS IN CATTLE WITH EPHEMERAL FEVER

- FEVER (104–107°F)
- ANOREXIA
- DROOLING
- SEROUS OR SERO-MUCOID NASAL DISCHARGE
- SEROUS OCULAR DISCHARGE
- WEAKNESS
- LAMENESS
- INJECTED NASAL MUCOSA & CONJUNCTIVA
- RECUMBENCY
- PARESIS - RARE
<table>
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<th>PN***</th>
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<td>+</td>
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<tr>
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<td>Inoculated intravenously with virulent EFV</td>
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<td>2.0</td>
<td>1:10</td>
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<tr>
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<td>Inoculated intravenously with virulent EFV</td>
<td>+</td>
<td>1.0</td>
<td>NT</td>
</tr>
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<td>NT</td>
</tr>
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<td>281</td>
<td>4 weekly inoculations with EFV MB 8</td>
<td>-</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>299</td>
<td>5 weekly inoculations with EFV BHK 10</td>
<td>++</td>
<td>4.75</td>
<td>1:3840</td>
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<td>56849</td>
<td>Australian convalescent serum</td>
<td>+</td>
<td>1.75</td>
<td>1:10</td>
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* Agar gel diffusion precipitin
** Neutralization index in MS cell cultures Log 10
*** 50% plaque neutralization titer in Vero cell cultures

*Sera obtained 14 days after last inoculation

NT not tested

A subcutaneous
REFERENCES

REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

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Venezuelan Equine Encephalitis

Extensive outbreaks of Venezuelan Equine Encephalomyelitis (VEE) have occurred in South America, and in Guatemala, El Salvador, Honduras and Nicaragua in Central America. On the 15th of October, 1969, the most recent outbreak of VEE was reported near Tampico, Mexico. VEE virus has been isolated from mosquitoes and wild rodents in south Florida. Also, hemagglutination inhibition (HAI) anti-bodies to VEE have been found in humans and rodents in the same areas and serum neutralization tests of paired serum samples from two women with a VEE-like syndrome demonstrated a significant rise in VEE neutralizing antibodies. The apparent absence of equine involvement may account for the low number of human cases, since it is generally accepted that horses must act as amplifying hosts before an extensive epidemic can occur. In this respect, VEE differs significantly from Eastern Equine Encephalomyelitis and Western Equine-Encephalomyelitis in which the horse and man are only dead-end hosts. Even though clinical VEE has not been reported in equidae in the United States, ecological conditions in the Gulf Coast area are similar to the ecological conditions existing in the Central American republics suggesting that the Gulf Coast area is vulnerable to an epizootic with this virus.

The United States (US) government assisted in combating the outbreak in Central America by providing about 575,000 doses of an experimental vaccine. Although the vaccine was developed for human use it appeared to work well in equines. The problems involved in production and use of the experimental vaccine were discussed at an interagency meeting in Washington, D.C., on September 25, 1969. It was the consensus of the experts attending that the vaccine is safe and effective, but needs further testing in equidae before it is licensed as a veterinary biological product. Since the supply of experimental vaccine is critically low and none is being produced, experts also urged that the USDA consider licensing the vaccine for production in the U.S. and sale overseas.

The Committee recommends that USDA approve the production of the
experimental vaccine in this country for sale outside the United States provided the vaccine passes the required safety, potency and return to virulence tests and provided there are ample safeguards to prevent escape of the virus from vaccine testing and production facilities.

**Rinderpest**

The Joint Rinderpest Campaign in Africa continues to make substantial progress. However, there appears to be an unfortunate let-down in maintaining vaccination of young susceptible animals in West Africa, and a number of small rinderpest outbreaks have been reported in West Africa during the past year. Apparently several countries are either unwilling or unable to maintain a vaccination schedule.

A rinderpest outbreak was reported in Iran, probably as a result of importation of infected slaughter animals from Afghanistan.

Rinderpest appeared in Kuwait in August 1968. The outbreak was immediately brought under control, and no cases have appeared since September 1968.

**Foot and Mouth Disease (FMD)**

The FMD situation in South America remains critical and the Inter-American Development Bank has approved loans to Argentina, Paraguay and Chile for FMD Control Programs. Loans to other South American countries are under consideration. The terms for loans do not provide for adequate coordinated international program planning and execution, adequate support for the Pan American Aftosa Center or adequate quality control of vaccines.

The worldwide FMD situation has been reported by the Vesicular Diseases Committee and, therefore, will not be covered by this Committee.

In a paper presented to a joint meeting of the Vesicular Diseases and Foreign Animal Diseases Committees, Dr. Taylor, of Plum Island, reported that the collared peccary, a native wildlife species of Southwestern U.S. and Mexico, is highly susceptible to rinderpest. Rinderpest in this species produced a fatal disease. The peccary was susceptible to Vesicular Exanthema of Swine (VES), Vesicular Stomatitis, FMD and hog cholera, but was not susceptible to African Swine Fever (AFS). The information reported by Dr. Taylor indicates that peccaries in the U.S. would have to be kept under close surveillance should rinderpest or the vesicular diseases appear in Mexico or Southwestern U.S. The entire report will appear in the proceedings. Dr. Taylor's report suggests the need for further studies on the susceptibility of native wildlife species to agents of foreign animal diseases. The Foreign Animal Diseases Committee commends Drs. Dardiizi, Taylor Yedloutschnig and the Plum Island Animal Disease Laboratory for this work and recommends that similar studies be conducted with other U.S. wildlife species.

**Besnoitiosis**

Besnoitiosis was demonstrated by histological evidence in skins of goats collected several years ago in Kenya. The only previously recorded case was in a
horse in 1963. The occurrence of the disease is now suspected in other species. In Rhodesia, besnoitiosis was recently diagnosed in cattle.

**Sheep Pox**

Sheep Pox was confirmed in sheep in Ceylon. Previously the disease was suspected, but not confirmed.

**East Coast Fever**

East Coast Fever has now been eradicated in Swaziland. The last case was recorded in March 1966. Law requires compulsory dipping of susceptible animals and examination of blood smears (from all dead and slaughtered bovines) for the presence of Theileria parva.

**Japanese B. Encephalitis**

Japanese B Encephalitis is markedly decreased in pigs in Japan. Hemagglutination inhibition antibody testing is required on all slaughter pigs. It has been shown that domestic swine can serve as a reservoir for Japanese B virus.

**Tsetse Fly**

Development of the sterile male technique for the control of the tsetse fly is being supported by the U. S. Agency for International Development, the International Atomic Energy Agency, the European Economic Community and the World Health Organization. Research and control programs are in progress or planned in the Lower Chad Basin, the Central African Republic, Kenya, Uganda, Tanzania and Zambia.

**Canine Idiopathic Hemorrhagic Disease**

The Foreign Animal Diseases Committee usually does not concern itself with diseases of dogs and cats. However, a condition designated as idiopathic hemorrhagic syndrome, believed to be tick transmitted, is reportedly causing losses in dogs in Viet Nam, Puerto Rico and the Virgin Islands, and the Committee believes information concerning this syndrome should be disseminated to U. S. veterinarians. The Committee is advised that investigators from the Division of Veterinary Medicine, Walter Reed Army Institute of Research report the consistent finding of Ehrlichia-like organisms in natural cases and in experimental dogs inoculated with specimens from affected dogs from widespread geographic areas suggesting that it may be the etiologic agent of this hemorrhagic disease.

**Recommendations**

The Foreign Animal Diseases Committee recommends that the Animal Health Division, Agricultural Research Service, in coordination with other interested agencies, initiate a program to prepare training films and other aids to be used in instructing veterinarians in the recognition, diagnosis and control of foreign animal diseases.
The Committee also recommends that the Association support a movement to obtain funds for the construction of a facility at the Plum Island Animal Disease Laboratory to train veterinarians in the recognition of foreign animal diseases which pose a threat to livestock and wildlife in the United States.
GROWTH OF EQUINE INFECTIOUS ANEMIA IN A NEW CELL SYSTEM


INTRODUCTION

In 1961, Kobayshi and coworkers (3) reported the growth of EIA virus in leucocytes maintained for long periods. Moore and coworkers (6) reported the growth of EIA virus in leucocytes obtained and maintained under a slightly different technique. Later, Henson and coworkers (2) have also verified this finding. Recently, Moore and coworkers (7) have developed a method for the continuous culture of the horse leucocyte. They have also reported the growth of EIA virus in this cell system (10). The purpose of this report is to give some of the characteristics of this cell culture virus.

MATERIALS AND METHODS

Cells

The method of cell culture from the continuous passage horse leucocyte were the same as reported previously (10) with some slight variation. A developed monolayer was first treated with 0.1% Pronase* at the rate of .5 ml. for every ml. of culture medium. After 3-5 min. the cell layer was removed by shaking the tube vigorously. The cells suspended in the pronase mixture were removed to a centrifuge tube containing 2 ml. of RPMI 1640 medium for each ml. of cell suspension. The cells were then centrifuged at 2000 xg. for 10 min. After centrifugation, the supernatant was removed and the cells were diluted with complete medium to a concentration of 300,000 cells/ml. The cells were then dispersed into appropriate containers.

The continuous culture horse leucocyte grown virus was also evaluated in the following cell cultures. PK 15 pig kidney cell line**, the MBKD bovine kidney cell line**, the McCoy cell line***, the KB cell line**** and the EBTR embryonic bovine trachea cell line*****. Three passages were made in all of these cells and

* Calbiochem, 3625 Medford, Los Angeles, California 90054.
From the Department of Veterinary Microbiology, College of Veterinary Medicine, Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas where Drs. Moore and Redmond are Professors and Dr. Katada is a Research Associate.
Supported in part by the American Quarter Horse Association through the Morris Animal Foundation, Denver, Colorado and Cooperative Agreement No. 12-14-100-9081 (45) with the Animal Disease and Parasite Research Division, ARS, USDA, Beltsville, Md.
**Obtained from the National Animal Disease Laboratory, Ames Iowa and maintained in our laboratory.
***Obtained from the Virus Laboratory, University of Texas Medical Branch, Galveston, Texas and maintained in our laboratory.
****Obtained from Flow Laboratories, Rockville, Md.
*****Obtained from American Type Culture Collection.
back titrated into the continuous passage horse leucocyte culture. In addition, an aortic endothelial cell line developed by Katada (4) was evaluated. All of these cultures were prepared by published standard methods.

Cell Culture and Medium

Cell culture medium varied from that previously reported in that RPMI 1640***** was used as the basal medium instead of medium 199. The present medium for the continuous passage horse leucocyte was RPMI 1640 - 50 ml., 10 ml. of 10% lactalbumin hydrolysate in distilled water and 40 ml. of sheep serum. After inoculation with virus the medium was RPMI 1640 - 75 ml., 10 ml. of 10% lactalbumin hydrolysate and 15 ml. sheep serum.

The medium for the growth of the aortic cell line was 70 ml. of RPMI 1640, 10 ml of 5% lactalbumin hydrolysate in distilled water and 20 ml. of sheep serum. After inoculation with virus the sheep serum was reduced to 10 ml. or in some cases to 0.

Virus Strain

Two different strains and various passage levels were used in this study.

Texas Strain. The Texas strain used in this study was isolated in primary horse leucocytes in 1964 and since that time has been passaged in primary horse leucocytes or stored at -20°C. The 8th passage primary horse leucocyte was passaged in continuous culture horse leucocytes for six more passages and filtered through a 50 μm filter. This filtered material was either passaged back into the horse leucocyte culture or into the aortic cell line. The 4th passage in the aortic cell line was used in this study for the comparison between the two cell lines.

Illinois Strain. The Illinois strain was isolated from the plasma of a horse inoculated into the continuous passage horse leucocyte. The 6th passage was filtered through a 50 μm filter and used in these studies.

Animal Inoculation Studies

Horse. The horse inoculation work was conducted in horses according to the instructions and protocol for horse inoculation tests published in the 71st annual meeting of the USLSA.

Guinea Pig. Two mature guinea pigs were inoculated intra-peritoneally with 1 ml. of cell culture fluid titering 10^5 TCID50 per 0.1 ml. Three passages were made by collecting spleen, blood and liver after a 10 day observation period, grinding with a glass tissue grinder, making a 20% suspension and passaging them into two more guinea pigs.

Mice. Day old and 10 day old mice were inoculated intraperitoneally with 0.1 ml. of cell culture fluid titering 10^5 TCID50 per .1 ml. Three passages were made by collecting spleen, blood and liver after a 10 day observation period, grinding with a glass tissue grinder, making a 20 percent suspension and passaging them into

*****Microbiological Associates, Bethesda, Md.
titrated into continuous passage horse leucocytes.

Embryonating Chicken Eggs. Five-6 day old embryonating chicken eggs were inoculated with 0.2 ml. of cell culture fluid titering $10^5$ TCID/50 per 0.1 ml. into the chorioallantois. When the embryos were 13 days old chorioallantoic fluid was collected and passaged into five more 6 day old chicken embryos. The embryos were examined at the 13th day for evidence of stunting and deformatives. After the third passage the chorioallantoic fluid was titrated into the continuous passage horse leucocyte culture.

Virus Characteristics

Growth. A growth curve was determined by making 10 fold dilution of daily aliquots of cell culture fluid from virus grown in both cell lines. Average endpoint titers were determined for both strains of virus in both cell lines. Indirect fluorescent antibody studies using a goat serum which had been inoculated with purified virus (9) was used to determine virus maturation.

Ultrafiltration. Cell culture fluids containing virus of each strain was filtered through Millipore******** membrane filters of 450, 220, 100, and 50 and 20 mu. pore diameters. The resultant filtrate of each was titrated in both cell systems.

Chloroform Sensitivity. Chloroform sensitivity was determined by the method of Feldman and Wang (1) in both cell systems and for both strains.

Nucleic Acid Type. Monolayers of both the continuous passage horse leucocyte and horse aortic cell line were inoculated with both the Texas and Illinois strains of virus. Medium containing 30 micro gms./ml. of 5-bromo desoxyuridine (BUDR) was added to one set of inoculated tubes while another set was inoculated with medium containing no BUDR. A strain of infectious bovine rhinotracheitis virus and a strain of parainfluenza 3 virus was treated in a similar manner as a positive and negative control.

RESULTS

Both strains of virus grew in the continuous passage horse leucocyte producing a 80-85% cytopathic effect (CPE) in 5-6 days postinoculation. (Fig. 1, 2 & 3). The Texas and Illinois strains were also isolated repeatedly from donor horse plasma. The Texas and Illinois strains were also adapted to grow in a horse aortic cell line with passage. No cell lines were capable of growing the virus as evidenced lack of CPE in each cell line and when titrated back into the continuous passage horse leucocyte.

The horse aortic cell line was a much more desirable cell to work with for virus characterization because of the ease of manipulation of the cell line. Virus has not been isolated to date from horses by using this system.

A basal medium developed for human leucocyte culture designated RPMI 1640 was superior to Medium 199 for both the horse leucocyte and aortic cells.

Of the animals inoculated (horse, guinea pig, mice and embryonating chicken eggs) only the horse as reported previously (10) showed evidence of infection. (Fig.

4) Also virus was reisolated only from the horse.

Fig. 5 shows a typical growth curve of the virus. Note that little or no virus was released until the fifth day with maximum titer reached on the 6th day. This held true for both strains of virus in both cell systems. Fluorescent antibody became weakly positive on the fifth and strongly positive on the sixth day postinoculation.

Fifty millimicron ultrafiltrates of both strains produced a CPE typical of that shown in Fig. 1 but 25 millimicron ultrafiltrates did not. Neither strain of virus grown in both cell lines was sensitive to chloroform.

5-Bromo-desoxyuridine derivative completely inhibited viral synthesis of both the EIA virus and IBR but did not inhibit parainfluenza 3. (Fig. 6)

**DISCUSSION AND SUMMARY**

A method for the continuous passage of horse peripheral leukocytes made it possible to recognize the effect of growth of EIA virus by its cytopathic effect. A field isolate had characteristics similar to one isolated and maintained in primary horse leukocyte culture. These two isolates did not show evidence of growth or infection in cell cultures or animals tested other than the horse.

When the virus was passaged in continuous culture horse leukocytes cells, cultural fluids and 50 μm ultrafiltrates of these fluids were capable of producing clinical signs and lesions compatible with EIA. The CPE effect was also reproduced by ultrafiltrates from a 50 μm membrane filter.

The virus strains were characterized by: 1) Small size 16-32 μm by filtration studies and 30 μm by electron microscopic studies (8), 2) Slow maturation (5-6 day) in both cell cultural systems, 3) Limited cell culture spectrum, 4) Chloroform resistant and 5) a DNA core as demonstrated by inhibition of virus synthesis by a halogen derivative of desoxyuridine, urynl acid uptake (8) acridine orange studies (11) and Fuelgen stains.

Electron photomicrographs and the above characteristics most closely resemble the small porcine DNA virus described by Mayr et al. (5).
Fig. 1: Continuous passage horse leucocyte cells stained with Giemsa stain. x 1000
Fig. 2: Continuous passage horse leucocyte cells 3 days after infection with EIA virus. Stained with Giemsa stain. Note the inclusion bodies typical of the parvovirus group. x 1000
Fig. 3: Continuous passage horse leucocyte cells 6 days after infection with EIA virus. Stained with Giemsa stain. x 650
Fig. 4: Horse infected with EIA virus grown in continuous culture horse leucocytes and filtered through a 50 μm filter. Note edema of prepuce and ventral abdominal wall.
Fig. 5: GROWTH CURVE OF EIA VIRUS

Days Postinfection

Titer of Virus (TCID/50 per 0.1 ml.)
### Fig. 6. STUDIES ON NUCLEIC ACID TYPE OF EIA VIRUS

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<td>+</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+ = CPE  
0 = No CPE
REFERENCES


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES


Equine infectious anemia remains a problem of major concern to the world's horse industry and scientific efforts to develop more definitive practical tests for the diagnosis of the disease continue. You have just heard a presentation on the "Growth of Equine Infectious Anemia Virus in a New Cell System" which promises further advancement in the area of improved techniques for the detection of the disease and which may provide the answer for which we have been striving for so many years. Until such time as this breakthrough occurs, however, we remain faced with refining those procedures now generally accepted in demonstrating the existence of EIA.

Specifically, though somewhat archaic, the animal inoculation test remains as the singular method of greatest widespread acceptance for confirming a diagnosis of "swamp fever". Also to a lesser degree the precipitin test continues to be used as a valuable tool in confirming disease existence. It is recognized that the precipitin test has certain limits but has made a measurable contribution toward our present knowledge of EIA and its control. We urge that research workers continue to seek improvements in aids to diagnose. When reporting precipitin test results back to the field we believe it would be advisable to give as much guidance as possible and to that end revise the precipitin test report form to specify the limitations of the test results and further declare the test as experimental. Distribution of test results shall be limited to appropriate livestock health officials and to other concerned parties technically qualified to interpret this test report information or lack thereof.

Under the present EIA prospectus procedures, horses found positive to the inoculation test are limited to disposition by either euthanasia or lifetime quarantine. Such restrictive measures tend to suppress the reporting of suspected cases or the disposition of valuable horses in those states where the guidelines contained in the prospectus are being followed. It may readily be seen that this tendency defeats those original aims of detecting and eventually eradicating EIA and thus results only in continuing spread of the disease. An alternative procedure was presented to the Committee for the disposition of animals found to be infected by horse inoculation methods which provides an option by the owner. This procedure is demonstrated in the following chart:
Your Committee recommends the adoption of this alternative as an amendment to the existing prospectus and guidelines on EIA.
The Committee was privileged to view a USDA produced 16-minute filming on EIA, directed primarily to lay audiences. This motion picture, now available through the Extension Services of the several states, as well as from the Department, for the benefit of you who wish to obtain copies for viewing is appropriately entitled "Equine Infectious Anemia." We wish to commend the Department of Agriculture and Texas A & M University which cooperated in its production on the film's content.

In the field of equine piroplasmosis, the Committee reviewed the results of treatment of horses for Babesia caballi and Babesia equi during the past year, and in the light of this additional work, recommends the revision of the prospectus included in the report of this Committee for 1968, as follows: Horses found positive to infection with Babesia caballi should be treated with Diampron at the rate of 4 mg/lb body weight intramuscularly for two consecutive days. Horses found infected with Babesia equi should be treated with Diampron at the rate of 4 mg/lb body weight intramuscularly for four consecutive days. Horses found infected with both species of Babesia should be treated with Diampron at the rate of 4 mg/lb body weight intramuscularly for four consecutive days. In the case of B. equi and dual infections, horses so treated should be proved by sub-inoculation to have been cleansed of the organism. Data substantiating the preceding recommendations is herewith submitted for inclusion as an attachment to the prospectus on equine piroplasmosis.

"It has been reported that recently B. equi infection has been shown to exist on the Island of Puerto Rico and St. Croix by sub-inoculation and demonstration of B. equi in the blood of recipient horses.

"During the period Fiscal Year 1965 through 1969 the complement fixation (CF) test was applied to 3,625 equidae using both B. caballi and B. equi antigens. The total number tested and total number of dual reactions are listed below:

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Total Tested</th>
<th>Dual by Excess 2+5 Classification</th>
<th>Percent Dual Reactions in Excess of 2+5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>1,081</td>
<td>57</td>
<td>5.2%</td>
</tr>
<tr>
<td>1968</td>
<td>921</td>
<td>32</td>
<td>3.4%</td>
</tr>
<tr>
<td>1967</td>
<td>174</td>
<td>3</td>
<td>1.1%</td>
</tr>
<tr>
<td>1966</td>
<td>472</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1965</td>
<td>562</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>203</td>
<td>18</td>
<td>8.8%</td>
</tr>
<tr>
<td>1969</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guantanamo Bay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1965, 1967 &amp; 1968</td>
<td>143</td>
<td>13</td>
<td>9.0%</td>
</tr>
<tr>
<td>St. Croix (U.S. Virgin Islands)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>69</td>
<td>28</td>
<td>40%</td>
</tr>
</tbody>
</table>
"Dual reactions between B. caballi and B. equi are in the range of 0-9 percent in all areas tested except St. Croix in the U. S.

Virgin Islands

"In regard to dual reactions in the classification of the EP status of animals, we suggest that the Committee consider adoption of the following policy:

A dual reactor is an animal which reacts to both CF antigens for EP. The reactions must be at least 3+5 to B. caballi and 2+5 to B. equi. (These are reactor levels established by Dr. Holbrook.)

"It is not possible at this time to determine by CF tests whether such a dual reactor is carrying the B. caballi or the B. equi organism.

"It is possible, but in our experience not probable, that the animal could have or could have had both organisms.

"Making a definite determination as to which organism is present requires subinoculation from the reacting animal prior to treatment. Subinoculations from equidae following treatment can only show whether B. caballi and/or B. equi are present after treatment and thus do not evaluate efficacy of treatment because such subinoculations do not show what the animal's status was prior to treatment.

"We recommend that all domestic horses showing dual reactions be treated for four days with Diampron, 4 mg/lb. body weight, followed by animal inoculation before release.

"Until the efficacy of such treatment is proved for B. equi, import horses should be rejected or be negative on subinoculation following such treatment.

"For comparative purposes all animals subjected to blood film examination should also be CF tested.

"In order to establish the efficacy of a treatment against B. equi the affected horses must be:

1. Proved by subinoculation to have B. equi.
2. Treated in a prescribed manner.
3. Proved by subinoculation to have been cleansed of B. equi."

The importation of horses from EP endemic countries and possessions continue to be a threat to the continental United States, and therefore remedial steps must be taken to preclude entry of infected animals on both an international and interstate basis. Therefore, the following resolutions are presented as a part of this report and for referral to the Committee on Nominations, Resolutions and Internal Affairs for adoption by this organization. (READ RESOLUTIONS)

A situation report on a disease of grave concern to the industry — Venezuelan Equine Encephalitis — was presented to the Committee. Extensive outbreaks of this disease have occurred during the past few months in several South and Central American countries and widespread vaccination of horses in these countries, utilizing vaccines produced for possible human protection, has taken place to thwart further disease spread. Apparently the incidence has peaked and may be on the decline according to the somewhat sparse reports that are being received from these countries. However, vigilance is being maintained in an effort to keep abreast of this potentially explosive situation. Additional concern, though, has been expressed with isolations of this virus in recent months from mosquitoes and rodents in South Florida. The existence of virus in these vectors and intermediate
INFECTIOUS DISEASES OF HORSES

hosts is a matter of great alarm to animal and human health officials, and with this in mind a sub-committee was appointed to undertake an immediate study of this problem directed toward the development of a prospectus encompassing VEE for completion by mid-1970. It is the understanding of our group that the Committee on Foreign Animal Diseases has discussed this matter and will make recommendations to this body concerning the vaccine problem. We wish to laud that committee for its foresight in this field as it is our opinion that the disease should receive high priority consideration.

Your committee wishes to direct attention here to the recent formation of the American Horse Council which now represents and speaks authoritatively for the entire horse industry. This council is dedicated to the promotion and virtual preservation of the nation's horse industry.

All breed registries and other major organizations involved in the horse industry support and participate in the council. The headquarters office has been established in Washington, D.C. Two of our own committee members also serve on the council's Board of Trustees. Mr. Ed Honnen as chairman of the board, and General Kester as chairman of a special committee for liaison with federal and state agencies. The first objective is to demonstrate the economic and social significance of the horse industry to the American public and specifically to state and federal legislators.

Of immediate concern is proposed federal tax legislation which if passed in its present form will drastically curtail not only the horse industry but also the purebred cattle industry and some other productive areas. Other stated objectives are (1) to promote and support equine research; (2) to support and help standardize quarantine and other disease regulatory programs; (3) to promote and facilitate the use of the horse for pleasure purposes, especially by the country's youngsters; (4) to promote the development of park and wilderness trail systems and other public facilities for the use of horse activities; and other measures in general to promote the popularity and usefulness of the horse in modern society.

It is good to know that the horse industry is now organized and capable of functioning on a par with other livestock industries. It should make our regulatory tasks in this area a bit easier. Although in its initial development, the group is apparently succeeding in unifying activities of the equine industry in its many facets, for which we extend our plaudits and invite their participation in the deliberations and activities of this committee.

RESOLUTION

WHEREAS, it is generally known that equine piroplasmosis (EP) exists in many countries of the world; and

WHEREAS, there is an extensive movement of horses from such areas into the United States; and

WHEREAS, there is evidence that horses imported from such areas have been found affected with the disease or infested with *Dermacentor nitens* ticks upon arrival in the United States; NOW, THEREFORE, BE IT

RESOLVED, That the Code of Federal Regulation, Title 9, be amended to require the following:
REPORT OF COMMITTEE

1. All equidae, except those born in Canada, including zebras, be freed of ticks in the country of origin, be required to have a USDA permit prior to leaving the country of origin, and upon arrival in the United States be inspected, found free of ticks and receive a precautionary treatment with a permitted tickicide.

2. Prior to being released for entry, such equidae be subjected to the CF test for EP and handled as follows:
   a. Animals not positive to CF test be allowed to enter if otherwise eligible.
   b. Animals positive to CF test with *B. caballi* antigen be allowed to enter following recommended chemotherapy.
   c. Animals positive to CF test with *B. equi* or both antigens be refused entry or be allowed to enter following approved chemotherapy and further followed by negative animal inoculation tests at owners expense and properly supervised and certified by a veterinarian of the ANH Division, U. S. Department of Agriculture.

RESOLUTION

WHEREAS, a high percentage of equidae in Puerto Rico and the U. S. Virgin Islands have reacted to the CF test for equine piroplasmosis (EP); and

WHEREAS, it has been proved by inoculation of blood from horses in both Puerto Rico and the U. S. Virgin Islands into recipient horses that both *Babesia caballi* and *Babesia equi* exist in both locations; and

WHEREAS, horses affected with EP and/or infested with *Dermacentor nitens* have been moved from Puerto Rico into several states; NOW, THEREFORE, BE IT

RESOLVED That the Code of Federal Regulations, Title 9, be amended to require the following prior to equidae being moved to the continental United States:

1. All equidae be treated to cleanse them of ticks and upon arrival in continental United States be inspected, found free of ticks and receive a precautionary treatment with a permitted tickicide.

2. Prior to moving from Puerto Rico or the U. S. Virgin Islands, all equidae be subject to CF tests for EP and handled as follows:
   a. Animals not positive to CF test be allowed to move if otherwise eligible.
   b. Animals positive to CF test with *B. caballi* antigen be allowed to move interstate following approved chemotherapy.
   c. Animals positive to CF test with *B. equi* or both antigens be allowed to move interstate following recommended chemotherapy and further followed by negative animal inoculation proving them to be free of EP.

3. All such treatments be at the owners expense, properly supervised, and certified by a veterinarian of the ANH Division, U. S. Department of Agriculture.
SHEEP SCABIES ERADICATION

An intensified sheep scabies eradication campaign was conducted in parts of 11 States during fiscal year 1969. This campaign involved the application of all available tools; i.e., inspection of all flocks in the selected areas, reinspection of previously infected or exposed flocks, inspection at concentration points, collection and submission of specimens from suspicious animals at slaughtering establishments, use of the maceration-flotation procedure, liberal use of dipping, epidemiological investigations, involvement of local groups, training programs within the respective States, and publicity campaigns.

A total of 19 flocks were found infected of which 18 were located within the selected area and the 19th in an adjoining county where an owner reported a suspicious condition to State officials.

Epidemiological investigations linked the 19 outbreaks into eight series of outbreaks. The investigations were used in the selection of the area for intensified effort during fiscal year 1970. This area involves parts of 10 States.

A total of 15 counties in four States are listed in the Sheep Scabies Infected-Eradication Area. In three of these counties, infection was found in both fiscal years 1968 and 1969; they are Clarke County, Virginia, and Chester and Dauphin Counties, Pennsylvania. Two counties have been removed from the Sheep Scabies Infected-Eradication Area during fiscal year 1970: Culpeper County, Virginia, and Christian County, Kentucky.

It is reasonable to believe, on the basis of the epidemiological investigations, that there is foci of infection remaining undetected in several States.

Inspections of sheep for scabies during fiscal year 1969 did not change appreciably from the totals for fiscal year 1968. During fiscal year 1969, 4,297,926 sheep were inspected at public stockyards and 10,265,723 at points other than public stockyards. These totals are three quarters of a million (721,406) less at the stockyards and half a million (524,018) less at other points. This total is less than half the inspections that were performed during fiscal year 1965.

COUNTIES IN INFECTED-ERADICATION AREA

Virginia - Clarke*
New Jersey - Camden, Salem, Monmouth
Pennsylvania - Chester*, Lehigh, Lancaster, Franklin, Dauphin*, Mifflin, Juniata
Kentucky - Ohio, Muhlenberg, Barren, Allen

*Placed in the Sheep Scabies Infected-Eradication Area during fiscal year 1968.

CHORIOPTIC SCABIES

Chorioptic mites were isolated in nine flocks with 841 sheep during fiscal year
1969. These flocks were spread across the country with two flocks located in Kentucky and one each in California, Illinois, Maryland, Oklahoma, Tennessee, and Texas.

CATTLE SCABIES

Psoroptic cattle scabies was disclosed in six herds in three States (Arizona, New Mexico, Texas) during fiscal year 1969. The six outbreaks are grouped into two series. One series involves five herds — one each in Arizona and Texas and three in New Mexico. The second series consists of a single herd in Webb County, Texas.

Epidemiological investigations enabled the connection of the five herds involved in the first series and the location of the herd involved in the second series after an infected animal was found at a stockyards at San Antonio, Texas. However, investigation failed to disclose a source of infection for either series.

It is apparent from reported outbreaks — four during fiscal year 1968 and six during fiscal year 1969 — and our failure to find the origin of the infection that foci of psoroptic cattle scabies remain undetected.

A total of 13 herds were reported infected with sarcoptic scabies and 225 herds with chorioptic scabies.

Two herds were reported with psorergatic scabies. One each in Minnesota and New Mexico. The Minnesota herd is the first reported outbreak of psorergatic cattle scabies outside the southwest.

The public concern regarding pesticides should be of interest to us. Every effort should be made to eradicate scabies before further restrictions make such eradication efforts more difficult.

Two dips remain on the permitted list for use against scabies infections — toxaphene and heated lime-sulphur. Heated lime-sulphur in a spray-dip machine was used against both chorioptic and sarcoptic cattle scabies in New York and Vermont. In Vermont, 11 herds infected with sarcoptic scabies were treated twice at 14-day intervals during calendar year 1968.

These herds were free of sarcoptic infection upon inspection during the summer of 1969. This is a decided improvement over a recommendation in an old Farmers’ Bulletin calling for four treatments at 6- to 10-day intervals and of warnings in reports that as many as six or seven treatments might be required to free a herd of sarcoptic infection.

We have treated six herds in Vermont with Ciodrin using a spray-dip machine. Each of these herds was selected as being infested with chorioptic scabies and lice. The herds will be examined at 180- and 360-day intervals to determine the efficacy of the treatment.

TICK ERADICATION

Active Program Continued in Texas: Efforts to keep Boophilus spp. ticks from entering Texas from Mexico, to prevent outbreaks from spreading, and to eradicate the ticks from infested premises continued. During fiscal year 1969 in the Buffer Zone adjacent to Mexico and under Federal quarantine; 33,268 lots of 775,724 livestock were inspected for ticks and 14,731 lots of 79,103 were dipped. Outside
the quarantined area 15,775 lots of 354,099 livestock were inspected and 1,227 lots of 25,845 dipped.

Thirty-two tick-infested herds were found in the quarantined area and 15 infested herds found outside the quarantine area. Infested herds were found as far away as a feedlot in San Patricio County; however most of the infested herds outside the quarantined area were in two areas of Dimmit County.

The number of infestations outside the permanently quarantined area was greater than had occurred for many years and caused considerable concern. Although historically the tick eradication program in Texas included only cattle and horses, during the past year several wild deer in Dimmit County were found to be infested with \textit{B. annulatus}. USDA, ARS, Entomology Research Division workers demonstrated that \textit{B. annulatus} could complete its life cycle on deer.

\textit{Eradication of Amblyomma variegatum ticks from St. Croix, U.S.V.I.}

When exotic \textit{A. variegatum} ticks were found on St. Croix an immediate cooperative eradication program began. Livestock on the 7 infested premises were dipped in 0.125 percent coumaphos (Co-Ral) at 7-day intervals until August 1968 when the dipping was continued at 14-day intervals. For ground treatment (from jeeps or by USAF C-123 aircraft) 80% sprayable carbaryl (Sevin) was applied every 3 weeks at the rate of 2 lbs. active ingredient per acre. Ground treatment was discontinued in December 1968.

The last collection of \textit{A. variegatum} had been in April 1968 and overall results encouraging. However, on August 7, 1969, a single male \textit{A. Variegatum} tick was found at the slaughterhouse on a bull from the eastern part of the island, the 7 infested premises being on the western end. No additional ticks have been found.

During FY 1969 on St. Croix the following were inspected/dipped:

- Cattle 36,674/36,701;
- sheep 9,667/9,667;
- goats 9,219/9,219;
- horses 1,996/2,014;
- donkeys 0/3;
- mules 0/2, swine 9,669/9,669;
- dogs 972/972;
- cats 2/0;
- rabbits 16/0;
- chickens 233/0;
- ducks 35/0; and deer 17/0.

\textit{Other Items of Interest}

Effective July 24, 1969, 9 CFR Part 72 was amended to recognize coumaphos (Co-Ral) 25 percent wettable powder (used at a strength of 0.20 - 0.25 percent) as a permitted dip. Dioxathion (Delnav) was similarly recognized at a strength of 0.125 - 0.175 percent effective December 4, 1968.

The Federal quarantine was lifted from Puerto Rico on August 28, 1968. No \textit{Boophilus} spp. ticks had been found on Puerto Rico since December 1952.

During calendar year 1968 exotic ticks were collected from animals offered for entry into the United States as follows:

- Los Angeles, California - \textit{Amblyomma} spp. from a tapir and \textit{Aponomma exornatum} from a lizard.
- \textit{D. variabilis}, \textit{D. halli}, and \textit{Ixodes bicornis} from inanimate objects.
- Honolulu, Hawaii - \textit{Rhipicephalus} spp. from caines.
- New York Port or Clifton, New Jersey, Quarantine Station - \textit{A. nutalli} from hedgehogs.
**REPORT OF COMMITTEE**

*R. sanguineus* from canine.
*R. evertsi evertsi* from zebra.
*Hyalomma* sp. from equine and partridge.

Houston, Texas - *R. sanguineus* from canine.
South Carolina - *D. spp.* from inanimate object.

Cattle, horses, or inanimate objects offered for entry from Mexico were found infested with *A. cajennense, A. maculatum, A. inoratum, Argus persicus, Boophilus annulatus, B. microplus, D. albipictus, D. nigrolineatus, D. nitens, D. occidentalis, D. variabilis, Ixodes pacificus, I. scapularis, O. megnini,* and *D. spp.* from a rabbit.

**UNITED STATES ANIMAL HEALTH ASSOCIATION**

**SCREWWORM PROGRAM**

During the first two quarters of fiscal year 1969 screwworm infestations were in greater concentrations in the southwestern states than at any time since the early days of the program in 1962 and 1963. This heavy concentration of screwworms was caused primarily by favorable weather conditions for the propagation of the fly. During fiscal year 1969 there were 9,204 laboratory-confirmed cases of screwworms in the four states adjoining Mexico; however, screwworms were limited to these four states and were prevented from migrating into other areas.

Because of the mild winter weather and the very favorable screwworm environment, it was expected that the spring of 1969 would be one of the worst on record. To prevent this techniques were modified by increasing production of sterile flies in early March, anticipating migratory patterns of fertile flies by releasing sterile flies over areas where screwworm normally occur, before the arrival of the fertile flies. In addition, both in Mexico and the United States intensified efforts were conducted on epidemiology. In spite of mild winter weather the screwworm program received considerable assistance from weather as the late spring and summer was extremely hot and dry. All of these factors contributed to the lack of anticipated heavy infestations of screwworms within the United States.

From January 1 through September 2, 1969, the sporadic outbreaks were limited to 204 laboratory-confirmed cases. There have been several weeks during late July and August in which no screwworm cases were reported in the United States. This lack of cases during this period of the year has never previously occurred although there is a normal mid-summer decrease in the number of cases. With our present techniques we hope to enter into the fall and winter with a minimum of sporadic outbreaks in the United States and keep screwworms down to a minimal level.

During fiscal year 1969 a total of 7.287 billion screwworm flies were released.

The ultimate solution for avoiding various degrees of seasonal infiltration of screwworms into the United States is to extend eradication into Mexico and establish a barrier of sterile screwworm flies at the Isthmus of Tehuantepec. Through a feasibility study directed and funded by the 89th Congress, the Department has determined that such a program would be both technically feasible and economically advantageous to this country.
PESTICIDES AND ECONOMIC POISONS
IN THE FOOD CHAIN

by

William B. Buck, D.V.M.

In recent years, drugs, pesticides, nutrient substitutes, and other chemicals have been added to animal feeds in an effort to more efficiently and economically dispense these agents to livestock and poultry. Also, many feed companies distribute fertilizers and agricultural chemicals not intended for mixing in feeds. With the advent of these practices have come problems, either due to human error or otherwise, which have resulted in losses for which the manufacturer is liable; and beyond that, there has been chemical contamination of the public meat supply in the form of residues resulting from excessive exposure of animals to pesticides and drugs.

There are three problems which we frequently encounter at the Iowa Veterinary Diagnostic Laboratory. These problems include the contamination of animal feeds with granular and powder formulations of pesticides, copper toxicity in sheep resulting from the feeding of cooper but no molybdenum in complete feeds for sheep, and finally the use of arsenic as a herbicide. In each of these problem areas, there has been great economic loss to livestock producers; but more importantly, there is a potential public health hazard in each case.

PESTICIDES IN ANIMAL FEEDS

Accidental incorporation of massive levels of insecticides in animal feeds is a primary source of the pesticide problems in livestock. This may occur as a result of misidentification by the farmer, who has previously stored granular or powder insecticides in the feed area and subsequently added it to a grain mixture, thinking it to be minerals; or it may occur as a result of error by the local elevator and even possibly by the feed manufacturer. In Iowa alone during the past three years, we have investigated and documented on the average of two such episodes per month involving corn and soybean insecticides including aldrin, heptachlor, diazinon, thimet, and other organochlorine and organophosphate insecticide compounds. The massive contamination of animal feeds naturally results in acute death of some of the animals being fed the mixture; but, more importantly, extremely high levels of insecticide may be accumulated in the fatty tissues and remain there at the time of slaughter for human consumption. Our laboratory has documented cases in which carcasses contained between 40 and 50 ppm insecticide in the fat of beef slaughtered for human consumption.

Oft times a large number of animals may be killed as a result of a massive exposure. These animals may contain 2-3,000 ppm insecticide in their rumen contents. The rumen of a 1,000-pound steer may contain as much as 200-250 pounds of feed. One can see that the use of several animals that have died of massive pesticide exposure for tankage, which subsequently will be fed to poultry, swine, and perhaps dairy animals, is a potential source of insecticides in eggs, pork, and milk. We have also investigated and documented such cases in swine in which
massive doses of insecticide have been mistakenly added in place of a mineral mixture, resulting in a heavy death loss plus contamination of the meat slaughtered for food.

A specific example is as follows: An Iowa farmer had 95 head of 550-pound steers in a feedlot. He purchased feed from the local elevator which included corn cobs, urea, and cracked corn. The next morning following delivery of feed on the previous evening, the farmer went out to find 35 head down in the mud dying in convulsive seizures with many others in various stages of convulsions and central nervous system involvement. Thirty-six died and the remainder recovered and were fed out for slaughter. Chemical investigation revealed 2,000 ppm aldrin in the stomach contents of the dead animals. The fatty tissues contained up to 58 ppm dieldrin in those animals that died. In tracing back the sequence of events which led to the massive contamination of the feed, it was found that the local elevator stored a large pile of corn cobs in a warehouse in which a partition holding the cobs in place consisted of a stack of 50-pound bags of 20 percent aldrin granules. In scooping up the corn cobs with a tractor, the operator had inadvertently broken the bags of aldrin releasing the granules into the cobs. Needless to say, the local elevator was responsible for the damages. Not only was there a severe death loss and a setback to the affected animals, but perhaps more importantly we know that 36 carcasses containing high levels of aldrin were processed in tankage for animal feeds. In addition, we know that the animals that recovered and were subsequently slaughtered for human consumption after several months, contained significant levels of dieldrin, the epoxide of aldrin.

Such episodes as the one just related commonly occur in our state, and there is evidence that they occur throughout the cattle and swine feeding states. These accidents always get widespread publicity by news media. They create a distrust by the consuming public and give the use of agricultural chemicals and drugs a black eye. It is our contention that if these episodes could be reduced, we would go a long way toward eliminating the fear that the public has of using pesticides and other chemicals, which are so necessary for the production of food and fiber. We, therefore, are recommending that hazardous insecticides prepared in granular or powdered form for agricultural purposes, be identified by the incorporation of a dye maker, such as charcoal, graphite, or some other readily identifiable material that would immediately show up if it were incorporated in animal feeds.

The Agricultural Chemical Committee at Iowa State University has recommended to the Iowa State Legislature that the Department of Health, Education and Welfare be requested to hold a hearing on the feasibility of color-identifying granular and powdered insecticides prepared for agricultural purposes. Such a practice would help eliminate the opportunity for massive exposure of livestock and certainly would help the feed manufacturer prevent accidental contamination of his product. Support of this recommendation is needed by the U.S. Animal Health Association.

COPPER-MOLYBDENUM IMBALANCE IN ANIMAL FEEDS

The second problem I wish to discuss is one that puts the feed manufacturer against the Food and Drug Administration with the farmer in between. It is a man-made problem. Copper is recognized as a safe and necessary ingredient for
animal feeds. Molybdenum is not recognized as safe and necessary and by law cannot be incorporated in animal feeds. Yet, years of clinical veterinary experience and numerous scientific reports unquestionably indicate that a balanced ratio between copper and molybdenum is necessary for most livestock, especially sheep, and to a lesser extent, cattle. The fact is that copper-molybdenum-sulfate ion metabolism and excretion are closely interrelated. If sheep, for instance, are fed a diet containing a normal copper level (8-11 ppm) but with no molybdenum, copper toxicity may result. Therefore, when a vitamin-mineral preparation containing copper but no molybdenum is added to the ration, the copper level of the ration may be elevated to as much as 25-35 ppm; and since the natural molybdenum level in feed is usually low (1-2 ppm), copper poisoning may occur. On the other hand, excessive molybdenum in the presence of low or even normal levels of copper may result in molybdenum poisoning.

Presently, mineral-vitamin preparations are being combined in complete feeds for sheep. We have encountered at least 20 episodes in Iowa this year in which copper poisoning has resulted. Reports from other states and Canada indicate that this problem is widespread. Usually expensive lambs for show purposes, or ram lambs on tests are involved. We have seen the problem in the Iowa State University sheep flock for several years. Often ram lambs worth $500-1,000 are lost. Usually only about 5 percent of a flock are affected, however. Clinical signs and post mortem findings are classical of copper poisoning: rapid onset, weakness, off feed for 12-24 hours, "bloody" urine, yellow discoloration of the tissues, and severe hemolytic anemia. Mortality is very high and occurs within two to three days.

The Bureau of Veterinary Medicine is aware of this problem, but the obstacles preventing its correction go beyond them to other bureaus in the Food and Drug Administration. Because scientific reports indicate that molybdenum added in excess without copper in balance will cause molybdenum poisoning, it has been concluded that molybdenum is not safe and necessary. The fact is ignored that if molybdenum had been added in natural balance with copper (1 part molybdenum to 6-10 parts copper) no toxicity would have resulted. Also ignored is the fact that if copper were added to the diet without molybdenum or SO₄⁻ being present, copper poisoning would result. There are numerous documented studies confirming this phenomenon. Thus, by itself, copper should not be considered safe and necessary. However, in balanced combination with molybdenum, both are safe and necessary.

Failure to recognize molybdenum as safe and necessary is based partly on the fact that molybdenum is accumulated in tissues and eliminated in milk when given in excessive levels in the absence of copper, thus creating a potential public health hazard. I should point out, however, that under present regulations, sheep consuming a diet containing copper but no molybdenum may have copper levels of 2,000 ppm or greater in the liver, which could also be hazardous. This problem could be solved by adding molybdenum and copper to animal feed at a ratio of about 1 part molybdenum to 6-10 parts copper.

Another possible solution would be to add copper to animal feeds only in the form of copper sulfate. The sulfate SO₄⁻ ion facilitates the excretion of copper. Therefore, copper in combination with sulfate is less toxic.

If this problem is not corrected, attempts will undoubtedly be made to hold
the feed manufacturers liable. I favor such action to some extent. The FDA may be more responsive to suggestions by feed manufacturers. Such action also could be a means by which the public would become aware of this regulatory error, and may result in its correction.

In addition to copper-molybdenum problems, there are other trace minerals for which the regulations may need to be revised. Selenium is a good example—necessary at low levels but extremely toxic at high levels.

ARSENICAL HERBICIDES

During the past five years, the Iowa Veterinary Diagnostic Laboratory has encountered numerous problems associated with the common practice of using commercial preparations of arsenical herbicides. There are many preparations presently being sold, ranging from crab grass control for lawns in towns and cities, to control of weeds along country roads, school yards, highway right-of-ways, and for removal of thistles and other noxious shrubbery. Commercial preparations containing arsenic recommended as herbicides contain up to 50 percent metallic arsenic. Many preparations contain 40-50 percent arsenic trioxide or sodium arsenite. Others contain arsenate of lead. These preparations are very toxic to pet animals, livestock, birds and humans.

The following examples of poisoning in animals and potential poisonings in humans have occurred:

1. (1969) The back yards of several homes in West Des Moines adjoin to form a small 1-2 acre pasture in which several of the families maintained pleasure horses. One of the back yards was treated with a crab grass control containing 47 percent arsenic trioxide and 3½ percent arsenate of lead. Approximately three weeks later, after several rains and after the lawn had been watered, the grass was clipped with a lawn mower and the clippings thrown over the fence to the horses. The horses readily ate the clippings and within a period of four days a total of five horses were killed. The grass clippings contained 6 percent metallic arsenic.

2. (1969) A janitor of a local high school used a commercial preparation of sodium arsenite as instructed, spraying the periphery of a football field including a fence row adjoining a cattle pasture. A few hours later the cattle ate the grass contaminated with arsenic, eating a strip approximately 8 inches wide next to the fence. A total of five cows were killed and four were affected but recovered.

3. (1965) A care-taker for a cemetery sprayed the fence row with a sodium arsenite commercial preparation allowing the spray to drift for a few inches over into an adjoining cattle pasture. A total of 15 dairy cows were killed. The dairy-man and the farm family consumed milk from an affected cow and arsenic poisoning was diagnosed in one member of the family.

4. (1968) A commercial crab grass control formulation containing arsenic trioxide was applied in the fall of the year to grass in a backyard. The next spring, at least four months later, the grass was clipped and the clippings thrown over the fence to calves which subsequently ate the clippings resulting in the death of several animals.

In every above instance, the level of arsenic in the grass after the application of the arsenical herbicides was extremely high, up to 6 percent (60,000 ppm metallic
arsenic). It has become obvious that the arsenical herbicides are extremely toxic to animals. In one case a dog was poisoned after eating some of the blades of grass following the application of an arsenical crab grass control preparation. Livestock apparently like the taste of arsenic and will seek out soil and plants sprayed with an arsenical preparation. Invariably this results in death to many animals. Humans are susceptible to arsenic and it is quite feasible that persons working with arsenical herbicides could be poisoned. In addition, children playing in backyards to which an arsenical crab grass control herbicide recently has been applied, could be poisoned. It is feasible that a small child could drop a candy sucker into the grass, pick it up, and enough arsenic be present to poison the child. Such occurrences would not be immediately obvious to a physician and severe illness and death could occur without the physician being aware of the cause. Such cases can only be diagnosed with the aid of analytical chemical procedures, such as are available at the Veterinary Diagnostic Laboratory at Iowa State University.

Recommendations

It is our recommendation that arsenicals be banned for sale and use as a herbicide. There are many good organic herbicides that can be used. Apparently, the only reason for the use of arsenical herbicides is that they are less costly than most presently recommended organic herbicides. Frequently local governments, such as school boards, county supervisors, and perhaps the state highway commission utilize the arsenical herbicides. Also, certain commercial firms use arsenicals to kill weeds in areas such as electrical highline right-of-ways and substation plots. The general population is not aware that arsenical herbicides are extremely toxic to humans and animals whereas most other organic herbicides are relative nontoxic.

A lively and lengthy discussion followed Dr. Buck's presentation. As a means of preventing accidental contamination of animals to be used as human food with potentially deleterious chemicals the Committee on Public Health and Radiological Fallout recommends that the United States Animal Health Association request that the Consumer Protection and Environmental Health Administration of the United States Department of Health, Education and Welfare, the Agricultural Research Service of the United States Department of Agriculture, and the Division of Research of the United States Department of Interior hold hearings on the adoption of methods of color identification of granular and/or powdered insecticides which might be used for agricultural purposes. The committee on Public Health and Radiological Fallout recommends that the United States Animal Health Association transmit a copy of the portion of the committee's report on Pesticides and Economic Poisons in the Food Chain and the proceeding recommendation to the Secretary, United States Department of Health Education and Welfare as a statement of the associations support of his committee on the development of a national policy on pesticides in relation to food, the environment and the public health.

Dr. Parker summarized the number of cases of human listeriosis voluntarily reported to the National Communicable Disease Center for 1968. Cases reported increased from 60 in 1967 to 105 in 1968, at least 24 of the 1968 cases were fatal. The greatest incidence was in infants of less than one year of age in whom a case
fatality rate of 24% was observed. In patients over 40 years of age the case fatality rate was 77%. Only four cases with no fatalities could be identified as belonging to the 2 to 40 year old age groups. Onset, judged by date recorded or date of first culture, occurred in 60% of the cases during the summer and early fall months.

Attention was called to the professional team work necessary in defining sources of infection of many of the zoonosis. Examples of the current cooperative attitude are the policies in effect for tracing meat borne outbreaks of food poisoning beyond the packing plant to point of origin and the developing program for tracing brucellosis to herds foreign when cases in animals or man are reported.

The meeting adjourned at 4:30 P.M. The Committee respectfully submits this report for consideration and acceptance.
Demodectic Mange

The committee had an opportunity to hear from Mr. Gerhard Boerger, in the tanning industry and to observe and to study microscopically an impressive number of cattle hides he brought for us to see as examples of damage due to demodectic mange.

Mr. Boerger reported that 90-98 percent of the hides received at the tannery he represents were damaged to greater or lesser extent due to the animals being infected by demodectic mites. This damage results in an approximate loss of 25 percent in the values of tanned hides. The Tanners Council has estimated our annual losses from demodectic mange in the United States to be up to 10 million dollars.

Although damage to hides is well documented there is no film evidence that the infestation causes measurable unthriftiness or loss of production in dairy or beef cattle.

We are unable, at this time, to recommend a means of treating cattle having demodectic mange and research works are pessimistic that an effective treatment will be forthcoming in the near future.

The committee recommends that research on demodectic mange be continued and if possible expanded.

Cattle Grubs

Your committee discussed the interest expressed by some livestock groups in Cattle Grub Control. Representatives of the committee will discuss the matter with members of the cattle industry at the meeting of the National Cattlemen’s Association to be held in Washington, D.C., January 1970.

Face Fly and Bluetongue

This committee supports the positions of the Committee on the Infectious Diseases of Cattle that research be intensified in the face fly (as a spreader of infectious keratitis) and studies of bluetongue vector.
Exotic Animals and Ectoparasites

The committee again recommends that import regulations be strengthened to require prior permits for certain animals not now requiring permits, that exotic and zoo animals be freed of ectoparasites prior to being moved to the United States.

Registration of Miticides

The committee calls attention to the association’s resolution that compounds used externally against ectoparasites, including all species of mites, be permitted registration as pesticides rather than as drugs and urges that letters to this effect be directed to appropriate agencies within the U. S. Department of Agriculture and to the Department of Health, Education, and Welfare.

Summaries of Fiscal Year reports on sheep scabies, cattle scabies, tick, and screwworm eradication are being submitted for inclusion into the published proceedings of the association.

Inasmuch as there were 19 outbreaks of sheep scabies found last year, a considerable increase over the previous year, the committee urges that we do not let up our fight against this disease until the effort to eradicate it has been completed.
CHEMOTHERAPY OF NATURAL AND EXPERIMENTAL EQUINE PIROPLASMOsis


INTRODUCTION

The antibabesial activity of drugs such as quinuronium sulfate (Kikuth, 1935), phenamidine and pentamidine (Lourie and Yorke, 1939) and Berenil (Enigh and Reusse, 1955) was first investigated using puppies infected with Babesia canis. Lucas, 1960, described the action of a new antibabesial compound 3:3 - diamidinocarbanilide di-isethionate, M & B 5062A. This new agent was found to show good activity at a dose of 5 mg/kg in splenectomized calves infected with B. divergens. Ashley et al (1960) found that amicarbalide was markedly less toxic than quinuronium sulfate and Beveridge et al. (1960) reported that the local and systemic tolerance of amicarbalide when used at the rate of 5, 10 and 20 mg. per Kg. body weight was good, and that it was effective in treating cases of B. divergens infection in the field.

Amicarbalide, as used in these trials, was a stable, aqueous solution of the di-isethionate.

\[
2\text{HOCH}_2\text{CH}_2\text{SO}_2\text{H} \\
\text{HN} = \text{CNH}_2 \\
\text{NH}_2\text{C}=\text{NH}
\]

3:3 diamidinocarbanilide di-isethionate

This agent is a white crystalline solid, which dissolves rapidly in water up to a concentration of nearly 100% w/v at 25°C. Preservative 0.1% w/v chlorocresol. The copy of the proposed label and dosage recommendations is attached.

In other countries, babesiacidal agents would be used for treatment of clinical cases at a dosage which would reduce the acute parasitemia and death losses. However, it is not desired to clear the infection but instead to establish adequate antibody protection along with the premunized infection to withstand field challenge.

From the Department of Veterinary Science, University of Florida, Gainesville, Florida. W. W. Kirkham is now located at Purdue University, LaFayette, Indiana. Published with the approval of the Director of the Agricultural Experiment Station as Journal Series.

These studies were supported in part by a grant No. 12-14-100-7158 from the Animal Disease and Parasite Research Division, ARS, USDA.

The authors express their appreciation to Dr. C. L. Campbell, State Veterinarian, Division of Animal Industry, Florida's Department of Agriculture and to Dr. J. B. Healy, Veterinarian in charge, ADE, ARS, USDA, Jacksonville, Florida, for their cooperation and support in these studies.
Research in Florida was directed toward evaluating chemotherapeutic agents for safety and effectiveness in eliminating the "carrier" cases of the several agents used previously in cattle basesiosis, three were selected as having promise; these were Berenil, Phenamidine and Diampron. Whereas, experimental infection B. caballi of both splenectomized and intact horses caused mortality of only 7 percent when not treated, 51 of 63 splenectomized and 8 of 18 intact horses died when given carrier or acutely infected B. equi blood.

Berenil given intramuscularly at 5mg/lb. and 1 to 4 doses cleared 20 of 25 B. caballi cases. Phenamidine, when given at 4 mg/lb. and repeated 1 to 3 times (total 2 to 4) at 1 or 2 day intervals was effective in eradicating the carrier status of B. caballi infected horses but was less effective in B. equi infected animals. Five of 20 animals receiving 4 doses were cleared but 9 of the 20 died due to infection or drug toxicity. These animals showed anorexia and icterus; at necropsy, the lesions included severe hepatitis along with intestinal hemorrhage.

Diampron was evaluated for safety and effectiveness against B. caballi and equi infections in experimental and clinical cases. Naturally occurring cases were identified by positive Giemsa or by the complement fixation test. Treatment was performed principally at a rate of 4 or 5 mg/lb. body weight and repeated on the following day. Blood was collected from these treated horses and injected into susceptible splenectomized horses or ponies housed in tight isolation facilities to insure freedom from extraneous exposure. The results presented in Table I demonstrate the high degree of efficacy for treatment and clearing of the carrier cases. Diampron was 99 percent effective in clearing naturally and experimentally B. caballi infected horses.

Freedom from the carrier state was demonstrated on repeated examination of Giemsa stained blood smears or in all experimentally infected and a representative group of the clinical cases by transfer of blood from the Diampron treated animals to splenectomized susceptible horses. After a proper period, these recipients of the treated animal's blood which remained negative to smears received known infected blood. They became positively infected animals; were then treated 2X with Diampron 4 mg/lb. body weight to clear the carrier state. Thus, many of the splenectomized ponies or horses were used repeatedly in testing the safety and efficacy of Diampron.

Earlier reports in the literature on the effectiveness of Diampron for treating babesiosis in cattle suggested that dosages somewhat less than the optimum were effective for treatment. In this research, a dosage of 1.7 – 3.0 mg/lb. repeated 2 or more times eliminated the carrier state in 24 of 28 horses or 85.6%. These animals were checked for clearance by transfer of blood after treatment.

Diampron, when given at a dosage of 4 mg/lb. body weight and for a total of 4 doses at 1 to 2 day intervals was effective in eliminating the carrier state in 5 of 20 B. equi infected horses. However, 9 of 20 of these horses were believed to have died due to either acute babesiosis or drug toxicity. They developed signs of anorexia and icterus, and at necropsy, lesions of severe hepatitis and intestinal hemorrhage were observed.

On the other hand, in another experimental trial, when given at a rate of
### CHEMOTHERAPY OF EQUINE PIROPLASMOSIS

#### DIAMPRON – SAFETY & EFFICACY vs. B. caballi in HORSES

<table>
<thead>
<tr>
<th>Dosage mg/lb.</th>
<th>Result Nos.</th>
<th>Cleared as Carrier</th>
<th>Not Cleared</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>68 ” 1X</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>45 ” 1X</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>23 ” 2X</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>23 ” 1X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15 ” 2X</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>10 ” 2X</td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6 ” 2X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5 ” 4X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5 ” 3X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5 ” 2X</td>
<td>225</td>
<td>223</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4 ” 2X</td>
<td>94</td>
<td>93</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>3 ” 3X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3 ” 2X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3 ” 1X</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>2.8 ” 2X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.6 ” 2X</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.5 ” 1X</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2.3 ” 3X</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
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<td>12</td>
<td>10</td>
<td>1</td>
<td>1</td>
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</tr>
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<td>1</td>
<td>–</td>
<td>–</td>
</tr>
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<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
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<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.0 ” 4X</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>0.7 ” 2X</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>361</strong></td>
<td><strong>345</strong></td>
<td><strong>8</strong></td>
<td><strong>9</strong></td>
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</tbody>
</table>
## TABLE 2

DIAMPRON EFFECTIVE IN CLINICAL B. caballi INFECTION

### Group A. Early Clearance of the Carrier State

<table>
<thead>
<tr>
<th>Tattoo</th>
<th>Date Treated</th>
<th>Dose/Day</th>
<th>Subinoculated</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1P1</td>
<td>4-26 &amp; 27-65</td>
<td>5.5 ml</td>
<td>5-1-65</td>
<td>Neg</td>
</tr>
<tr>
<td>6P2</td>
<td>10-1 &amp; 2-66</td>
<td>6 ml</td>
<td>10-8-66</td>
<td>Neg</td>
</tr>
<tr>
<td>6PS</td>
<td>10-29 &amp; 30-66</td>
<td>6.5 ml</td>
<td>11-3-66</td>
<td>Neg</td>
</tr>
<tr>
<td>6P4</td>
<td>10-29 &amp; 30-66</td>
<td>5.5 ml</td>
<td>11-3-66</td>
<td>Neg</td>
</tr>
<tr>
<td>6P7</td>
<td>3-18 &amp; 19-67</td>
<td>2.5 ml</td>
<td>3-21-67</td>
<td>Neg</td>
</tr>
<tr>
<td>7P1</td>
<td>6-8 &amp; 9-67</td>
<td>4.5 ml</td>
<td>3-12-67</td>
<td>Neg</td>
</tr>
<tr>
<td>7P2</td>
<td>6-26 &amp; 27-67</td>
<td>5 ml</td>
<td>6-29-67</td>
<td>Neg</td>
</tr>
<tr>
<td>7P3</td>
<td>6-26 &amp; 27-67</td>
<td>3.5 ml</td>
<td>6-29-67</td>
<td>Neg</td>
</tr>
</tbody>
</table>

### Group B. Demonstration of Persisting Clearance from the Carrier State

<table>
<thead>
<tr>
<th>Tattoo</th>
<th>Date Treated</th>
<th>Dose/Day</th>
<th>Subinoculated</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2P1</td>
<td>1-14-65</td>
<td>5 ml</td>
<td>3-4-65</td>
<td>Neg</td>
</tr>
<tr>
<td>3P5</td>
<td>1-14 &amp; 15-65</td>
<td>3.7 ml</td>
<td>1-14-65</td>
<td>Pos</td>
</tr>
<tr>
<td>4P1</td>
<td>6-22 &amp; 23-64</td>
<td>4.2 ml</td>
<td>8-13-64</td>
<td>Neg</td>
</tr>
<tr>
<td>4P2</td>
<td>4-22 &amp; 23-65</td>
<td>5 ml</td>
<td>6-22-65</td>
<td>Neg</td>
</tr>
<tr>
<td>4P4</td>
<td>10-21 &amp; 22-64</td>
<td>10 ml</td>
<td>3-4-65</td>
<td>Neg</td>
</tr>
<tr>
<td>5P2</td>
<td>3-30 &amp; 31-65</td>
<td>7 ml</td>
<td>3-22-65</td>
<td>Neg</td>
</tr>
<tr>
<td>6P6</td>
<td>11-22 &amp; 23-66</td>
<td>6 ml</td>
<td>1-27-67</td>
<td>Neg</td>
</tr>
<tr>
<td>7P4</td>
<td>8-14 &amp; 15-67</td>
<td>5 ml</td>
<td>10-2-67</td>
<td>Neg</td>
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<tr>
<td>R23</td>
<td>1-14 &amp; 15-65</td>
<td>4.5 ml</td>
<td>3-12-65</td>
<td>Neg</td>
</tr>
<tr>
<td>Red</td>
<td>6-22 &amp; 23-64</td>
<td>5 ml</td>
<td>8-13-65</td>
<td>Neg</td>
</tr>
</tbody>
</table>
4 mg/lb. body weight and repeated 3 times Diampron was effective in clearing the carrier status in 4 of 4 B. equi infected intact horses. An additional horse infected with B. equi was cleared with 2 doses at 4mg/lb. These results paralleled those observed in animals infected with B. caballi.

Since no signs had been observed in treating animals experimentally infected with either B. caballi or B. equi at the repeated 4mg/lb. dosage, it was determined that the influence of higher dosage rates would be examined. At repeated dosages of 8, 10 or 20 mg/lb., increasing signs of toxicity were observed in both intact and splenectomized B. equi infected animals.

Some have inquired whether subinoculation from Diampron treated horses would demonstrate both early and persistent clearance of the B. caballi carrier status. The data presented in Table 2 illustrates the safety and effectiveness of amicarbalide in clinical cases diagnosed as positive by blood smears.

**SUMMARY**

1. Diampron, at a dosage of 4mg/lb. body weight repeated in 24 hours has been shown to be effective in the treatment and elimination of the carrier state of B. caballi in horses.
2. At this same therapeutic dosage, it has been used effectively in treating clinical and experimentally induced B. equi infections in horses. Further work is needed to develop a safe and therapeutic dose for clearing the carrier state.
3. Experimentally induced B. caballi infections in intact and splenectomized horses, when treated with Diampron during the early stages or at peak parasitemia, were cleared of the infection.
4. Deaths have occurred in horses receiving Diampron at high, toxic levels or have been reported in a few horses with a persistent chronic, cycling carrier state or in those complicated by mixed infections.
DIAMPRON

Amicarbalide Isethionate

Diampron® is a brand name for di-isethionate salt of amicarbalide (3,3-diamidinocarbanilide di-isethionate) which has been demonstrated to have therapeutic value in equine babesiosis due to Babesia caballi and B. equi and to be highly effective in clearing the “carrier” state due to B. caballi.

INDICATIONS

Diampron® is indicated for the treatment and clearance of babesiosis, B. caballi, and for treatment of B. equi in horses.

DOSAGE AND ADMINISTRATION

In acute or severe cases with clinical signs inject only 4 mg/lb. or 0.8 ml./100 lbs. bodyweight intramuscularly. To clear B. caballi “carrier” cases, give 2 doses 4 mg/lb. bodyweight on consecutive days.

TOXICITY

Extensive laboratory and field trials have demonstrated safety of the drug when used as directed. In a few instances animals with babesiosis and concurrent infections or suspected of having severe or persisting hepatitis have shown transient systemic disturbances including uneasiness, colic, malaise, or ataxia.

A 50% w/v aqueous solution of amicarbalide isethionate in a 50 ml. multidose vial.

“Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian.”

Manufactured by May and Baker, Ltd.
Essex, England

Distributed by Rhodia, Inc.
New York, New York
REFERENCES

SAFE EFFICACIOUS DRUGS,
FDA'S RESPONSIBILITIES

We in the Bureau of Veterinary Medicine of the Food and Drug Administration are pleased to have this opportunity to discuss our responsibilities and concerns. It is our responsibility to apply the provisions of the Federal Food, Drug, and Cosmetic Act, as amended, to the drugs marketed for use in animals other than man. Our concerns relate to any possible health hazard which drugs used in animals may present. These are subjects I'd like to discuss with you today. Since we have a difficult assignment and one which few people completely understand, we welcome opportunities to explain what we are doing and why.

It is something over 40 years since Alexander Fleming's discovery of penicillin ushered in the new age of wonder drugs. Today, there are over 20 antibiotics widely used as drugs for the treatment of infectious diseases in man and animals and the search for new effective antimicrobials goes on with increasing intensity with each discovery that there are certain diseases that the existing drugs do not effectively control. The almost incomparable benefits from the use of antibiotics over the last 20 years are self-evident. There are, however, some perplexing, puzzling problems that are not as evident and, in some cases, varying inconsistencies which have surfaced as a result of this extensive utilization of antibiotics over the past 20 year period. As a result, concerned members of the scientific community as well as laymen have increasingly raised questions as to what relationship, if any, there might be between the use of antibiotics in large quantities in food animals and their continued usefulness in treating diseases of man and animals. The Food, Drug, and Cosmetic Act, as amended, specifically charges the FDA with insuring the safety and effectiveness of drugs and the purity and safety of foods. This is our responsibility.

In 1965, former Commissioner George P. Larrick, in response to the increasingly voiced concern, established an Ad Hoc Committee of outstanding scientists to review the veterinary medical and non-medical uses of antibiotics. After a year's intensive study and review, the Committee reported its concern regarding the non-medical uses of the drugs—particularly in the production of food and in food-producing animals. The Committee recommended that the FDA involve itself more deeply with the questions regarding these uses, and gather more information and data so as to assure better public health protection. The FDA concurred in the Committee's report and recommendations, and several specific actions were taken as a result.

First, in August 1966, the FDA notified the manufacturers of antibiotic drugs for veterinary use that they would be required to submit data showing the length of time antibiotic residues persisted in food from treated animals.

On the basis of the residue data submitted for the mastitis products and the results of FDA's own testing of these products, the Agency had to withdraw approval for some antibiotic preparations for use in the udders of cows because of

Presented by Dr. C. D. VanHouweling, Director, Bureau of Veterinary Medicine, Food and Drug Administration, at the Meeting of the United States Animal Health Association in Milwaukee, Wisconsin, on October 16, 1969.

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the persistence of residues in milk. For some years now, four day (96 hours or 8 milkings) has been the maximum time for milk discard approved for these products.

The incoming data on the persistence of residues of certain antibiotics — mainly streptomycin, dihydrostreptomycin, and neomycin — were indeed surprising. Slides 1 and 2 depict the unusual persistence of dihydrostreptomycin in the fat, liver, kidney, and at the site of injection in swine and cattle. Slide No. 3 demonstrates the elimination of aqueous penicillin in four days following parenteral injections. Slides 4, 5, 6, and 7, present similar data in regard to neomycin. This data, particularly the persistence of the dihydrostreptomycin and streptomycin, presented the agency with an unusual dilemma. The law specifically says in the case of a new animal drug, or a drug that would be covered under the provisions of the Food Additive Amendments to the Act, that unauthorized, unapproved residues shall be considered unsafe and, therefore, constitute adulteration of the product under the FD&C Act. In addition, the interstate shipment of an adulterated product is prohibited. Of course, the Act does provide for the safe use of food additives which, when fed in accordance with the directions for use will not leave harmful residues in the edible products of treated animals. In all cases except GRAS (Generally Recognized As Safe) items this requires the publication of a regulation providing for the use of the product, specific directions for its use, and when necessary, a tolerance for the residue in food and/or a period of time for the discontinuance of the treatment prior to slaughter — commonly referred to as "withdrawal time." The dilemma, of course, is obvious. The extremely long persistence of some antibiotics makes it impossible to write regulations with a reasonable withdrawal time. Almost everyone, I'm sure, agrees that a 75 or 90 day withdrawal period for the use of a drug in an animal is not practical.

For this reason it became necessary to propose some restrictions on the use of certain persistent antibiotics. In regard to streptomycin-dihydrostreptomycin, for example, we have published interim regulations providing for the continued use of these products parenterally in food animals, with a 30 day withdrawal period during which time any possible residue will be reduced to an extremely low level. During this interim, the Animal Health Institute is sponsoring research to demonstrate that a tolerance of 2 parts per million for dihydrostreptomycin can be safely established. This research includes not only tests in animals but in human volunteers as well. Human subjects will be fed low levels of dihydrostreptomycin and the resistance spectrum of the E. Coli inhabiting the intestines of these volunteers will be studied.

The legal restrictions of the Act in regard to the use of drugs and residues which become food additives, are based upon human health considerations. In our review of food animal drug uses and data, therefore, human health considerations are foremost in our minds. We, in the Bureau of Veterinary Medicine, must give human safety consideration top priority in our evaluation of drugs used in animals raised for food.

Obviously time does not permit an exhaustive discussion of the reasons for the public health concern, but they are as shown briefly on Slide 8 as follow:

(1) The allergic response that certain antibiotics, of which penicillin is the best known example, can cause in hypersensitive people. Even very small amounts in food have been known to cause such allergic reactions. Also,
the development of hypersensitivity through continued exposure.

(2) The toxicity, too, of such residues must be considered. It must be considered not only from the short-term standpoint but from the long-term standpoint as well, and this is why tolerance petitions usually require long-term toxicity studies prior to approval. Most of you, of course, are aware of the additional requirements of the so-called Delaney Clause which states that no residue of a carcinogen may be permitted in food derived from animals that have been treated with drugs.

In recent years increased concern has been expressed regarding the development of drug resistant organisms (slide 9). The knowledge of drug resistance transfer between bacteria, even of different species, naturally added to this concern. Questions were raised as to what possible consequence might be of gastro-intestinal bacteria in animals developing drug resistance and transferring such resistance to bacteria which might be ingested by human beings, and reinstigating the whole cycle in the human gut. In some cases, non-pathogenic bacteria or strains might be able to transfer such resistance to the pathogenic organisms, and thereby reduce the effectiveness of such drug therapy when employed in treating the disease in man. There is also the possibility of indirect transfer through food as well as the possibility of resistant organisms developing in the human gut, due to frequent exposure to drug residues in food. Admittedly, there is no hard data that this has occurred in the United States. But, by the same token, there is no hard data to demonstrate that it is not occurring and going undetected. For all these reasons we have had several meetings with representatives of other Federal Agencies, and the drug and feed industry associations to encourage research and investigations into the ecological effects of antibiotics used in food animals. There is some data being developed which appear to be reassuring, but nothing conclusive has yet been reported. Our Division of Veterinary Research has a cooperative epidemiological project with the Agricultural Research Service and its State cooperators, and the National Center for Communicable Disease Control and its State cooperative agencies. This is a small effort to determine whether or not epidemiological data can be collected which will show if there is a transfer of resistant organisms from animals to man, and concurrent animal and human disease problems on the farms.

Also, we have a contract with the College of Veterinary Medicine, University of Illinois, for the study of the public health hazard due to transferrable drug resistance of enterobacteria. The results of this work under the leadership of Dr. William Huber should provide some information relative to the ecological effects of these drug uses.

We are pleased that we were successful in securing a World Health Organization Fellowship for Dr. H. William Smith from the Animal Health Trust of England to visit this country to review the activities and investigations in the area of drug resistance and the transfer of resistance. Dr. Smith is a recognized authority in this field, was a speaker at the Symposium on the Use of Drugs in Animal Feeds sponsored by NAS/NRC in June of 1967 and most recently published an article in LANCET relative to the transfer of drug resistance between E. coli of animal and human origin to resident E. coli in the human alimentary tract. We hope as a result
of his visit to have the very best advice and counsel available and expect to receive some helpful suggestions from him in regard to our own investigational activities.

Since drugs that cause residues in food require regulations governing their use, it is well to re-emphasize some of the pertinent facts relative to these approvals with which we in FDA must always live. There is no such thing as a perfect drug, or for that matter a totally safe one. We continue, therefore, to watch the performance of a product once it is approved, and to take note of new scientific data that may be developed relative to its use. If there are developments which indicate that the approval previously given needs to be withdrawn or modified we, of course, must act accordingly. In this whole area of approval, and consideration of performance after approval, we have well defined responsibilities. If data shows that a hazard exists FDA must act promptly to curtail or restrict approvals, and even withdraw specific approvals which have been authorized because they are responsible for causing adulterated food. At the same time, we are well aware that what might be considered unfair or alarmist publicity concerning drug residues may harm both the consumers and the producers. Also, conceding that some risk is always involved, there are still many speculative questions that need to be discussed and answered. There are concerned scientists who are going to continue to ask questions. Research is going to have to be conducted, and facts are going to emerge. Pure speculation, without scientific basis, has no place in the decision reached in a scientific regulatory organization. But probing questions cannot be ignored. Without taking notice of these legitimate inquiries, science is in fact being ignored and public health protection may suffer. Human health must have top priority in our decision making when approvals and withdrawals are involved. Specifically — there are still questions being raised in regard to the large scale use of antibiotics and other drugs for certain purposes. In evaluating these and other theoretical questions, and in arriving at pertinent answers, FDA looks to the scientific literature and to the opinion of not only its own staff, but to scientists in industry and other agencies as well. When hard data are not available upon which to make decisions, we will and must rely on the best advice and counsel which we can get from recognized authorities in the areas of science and medicine and be guided by their recommendations.

As indicated previously, our charge is explicit with respect to food adulteration and human protection. We also have the responsibility for determining the efficacy of drugs submitted for approval as NDA’s. In reviewing recent pertinent literature concerning the improvements in animal feeding and production techniques — much of it based on the use of today’s powerful new veterinary drugs — we soon realize the impact of the decisions made by the FDA in regard to drug efficacy.

For example, it would be almost if not entirely impossible to have the flourishing broiler industry we have today without the availability of coccidiostats. Likewise it is difficult to picture cattle feeding without the use of diethylstilbestrol. These are only two examples of the significance of the use of drugs in animal feeds which will become even greater with the passage of time. However, the effectiveness of some drug uses are being questioned. We believe that, through an in-depth research effort, it will be possible to develop various criteria and models that will allow more objective and expeditious evaluations of the benefits of the various classes of drugs. Clearly this determination of efficacy is not an easy task. But, the
decisions must be made. And it is our belief that the task will continue to become even more difficult unless we begin — with you — and with industry — to develop more effective methods of measurement.

The proper control over the distribution and use of drugs in animals raised for food is obviously a highly complex task for FDA. It is even more complex if one weighs and considers the benefits are possible risk ratios and I believe they must be considered. In order to arrive at more objective, more scientifically accurate decisions, we in the Bureau are proposing a significant expansion in our research capability. These proposals call for considerable expansion in the area of drug testing models, as well as an expansion in the area of the effects and significance of drug uses and residues in animal tissues on the ecology.

I hope that the drug, feed, and livestock industries will also expand their contribution to this kind of scientific exploration so that together we can jointly answer more questions with facts based on solid data — rather than relying on informed opinions.

Clearly it is not an easy task to maintain the proper control over the distribution and use of drugs in animals, especially animals raised for food. We would like to further improve our communications with all similar groups, and it is through organizations like this and programs such as these that we are afforded these opportunities. With your understanding and our improved communication, I trust that we can depend upon your assistance and cooperation in discharging the difficult responsibilities assigned us under the Food, Drug, and Cosmetic Act.
### SUMMARY

Dihydrostreptomycin Residues in Hog Tissues and Body Fluids

Dose was 1.25 grams per 100 pounds body weight, given once intramuscularly

<table>
<thead>
<tr>
<th>Days After Dose</th>
<th>Hog No.</th>
<th>Muscle</th>
<th>Muscle Injection Site</th>
<th>Fat</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td>Fat mcg/gm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
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<td>6.95</td>
<td>1.70</td>
</tr>
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<td>6.5</td>
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<td>0.26*</td>
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*Extrapolated values
**SLIDE NO. 2**

**Dihydrostreptomycin Residues in Bovine Tissues**

After a single intramuscular injection of 1.25 grams per 100 pounds body weight

<table>
<thead>
<tr>
<th>Days after</th>
<th>Animal No.</th>
<th>Muscle</th>
<th>Muscle Injection Site</th>
<th>Muscle Fat Mcg/gm</th>
<th>Kidney</th>
<th>Liver</th>
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<td>1.89</td>
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<td>140</td>
<td>8.00</td>
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<td></td>
<td>6148</td>
<td>2.00</td>
<td>67.5</td>
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<td>120</td>
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<td>6160</td>
<td>2.50</td>
<td>22.0</td>
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<td>6157</td>
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<td>407</td>
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<td>10.5</td>
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<td>3.33</td>
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<td>517</td>
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<td>537</td>
<td>0.32*</td>
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<td>1.30*</td>
<td>-</td>
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<td>Neg.</td>
<td>1.50*</td>
<td>-</td>
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<td>511</td>
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<td>640</td>
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<tr>
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<td>691</td>
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<td>0.25</td>
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<td></td>
<td>6136</td>
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<td>Neg.</td>
<td>1.10</td>
<td>Neg.</td>
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*Extrapolated values
-No assays were conducted
**Penicillin in Beef Tissues**

Dosage: 3,000 units/lb. body weight

for four days via IM route

<table>
<thead>
<tr>
<th>Day</th>
<th>Fat</th>
<th>Muscle</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Injection Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>*</td>
<td>0.51</td>
<td>0.16</td>
<td>1.50</td>
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<tr>
<td>1</td>
<td>0.20</td>
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<td>1.01</td>
<td>0.72</td>
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<td>0.65</td>
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<tr>
<td></td>
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<td>&lt;0.10</td>
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<td>0.35</td>
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<td></td>
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<tr>
<td>3</td>
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</tbody>
</table>

* No zone of inhibition.
Neomycin Residue in Duck Tissues

Dosage: 5 mg/lb. body weight in drinking water for three weeks

<table>
<thead>
<tr>
<th>Day</th>
<th>Fat &amp; Skin</th>
<th>Mcg. Neomycin Base/Gm. of Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1</td>
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</table>

* No zone of inhibition
**SAFE EFFICACIOUS DRUGS**

**SLIDE NO. 5**

**Neomycin in Beef Tissue**

**Dosage:** 5 mg/lb. body weight daily for ten days via drench

**Animal:** 300 lb. beef calves

<table>
<thead>
<tr>
<th>Day</th>
<th>Fat</th>
<th>Muscle</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
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<td>-</td>
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</table>

* No zone of inhibition
- No assays were conducted
**Neomycin in Sheep Tissue**

**Dosage:** 5 mg/lb. body weight for four days - bolus  
**Animal:** 90 lb. lambs

<table>
<thead>
<tr>
<th>Day</th>
<th>Fat</th>
<th>Muscle</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
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* No zone of inhibition
Neomycin in Swine Feed: Tissue Residue Study

Dosage: Approximately 3.4 mg./lb. body weight for 21 days in feed
Animal: Swine weighing about 188 pounds

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* No zone of inhibition
- Assay not conducted
SLIDE 8 – POSSIBLE HEALTH HAZARDS ASSOCIATED WITH DRUG RESIDUES IN FOOD

I. Allergic Reactions
   A. In hypersensitive people
   B. Development of hypersensitivity

II. Toxicity
   A. Acute
   B. Chronic

III. Carcinogenicity
    A. Acute and chronic exposure

SLIDE 9 – RELATIONSHIP OF DRUG RESISTANT ORGANISMS TO HEALTH HAZARDS

I. Resistant Organisms in Animals
   A. Transferred to Man
      1. Through direct contact
      2. Through contaminated food

II. Development of Resistant Organisms in Man
    A. By continued Exposure to Drug Residues in Food
THE ROLE OF VETERINARY DRUGS
IN THE PRODUCTION OF WHOLESOME,
NUTRITIOUS AND ECONOMICAL FOODS

To supply an average American family of four, it has been estimated that the
American farmer must produce almost 6000 pounds of food each year. More than
3000 pounds of this total is meat, eggs, poultry and dairy products. Based on the
projected increase in population from a little over 200 million currently to 340
million people in the year 2000, our nation will need a 70% increase in the
production of these nutritionally important foods over the next 30 years.

While our needs are great, those of the rest of the world are much greater. In
their book Famine 1975, William and Paul Paddock predicted imminent starvation
in many developing nations and stated —

"The famines which are now approaching will not be caused by weather
variations and therefore will not be ended in a year or so by the return of
normal rainfall. They will last for years, perhaps several decades, and they are,
for a surety, inevitable. Ten years from now (1975) parts of the undeveloped
world will be suffering from famine. In fifteen years the famines will be
catastrophic and revolutions and social turmoil and economic upheavals will
sweep areas of Asia, Africa, and Latin America."

The consequences of the social turmoil which will result from this nearly
world-wide famine will affect not only those who fail to survive but the survivors as
well. In a recent television documentary on Africa, one segment dealt with a tribe
which many years ago had been forced out of their native lands into a desolate area
which produced very little food. According to the reporter, the members of this
tribe had no word for "love" and he never saw any indication of the emotion of
love or caring between husband and wife or mother and child. Children only two
years of age were customarily abandoned by their parents to fend for themselves
and find their own food as best they could. One shudders to think of mankind
having to endure under such circumstances.

Far fetched? Perhaps. Impossible? Probably not. And yet most of the world
steadfastly expects some miracle to avert the approaching spectre of world
starvation. Even the experts all seem convinced that the miracle will be brought
about — BUT NOT IN THEIR OWN FIELD OF COMPETENCY! Those knowledgable
in marine biology are sure that plant geneticists will be able to develop new
varieties of cereals which will flourish in the tropics, the arctic tundra, and the
desert; the plant geneticists are confident that chemists will find a way to produce
food from air, water, and coal; and the chemists have unbounded faith that the
oceans can supply an unlimited abundance of food. And so it goes — and the "man
in the street" believes only that an omnipotent "THEY" will come up with
something. To him the people in this room and the thousands of others in
government, academic, and industrial research institutions are "THEY". We, of

Presented at the Annual Meeting of the U.S. Animal Health Association, Milwaukee, Wisconsin,
October 16, 1969 by Dr. Henry S. Perdue, Ayerst Laboratories, 685 Third Avenue, New York,
N. Y. 10017 (Chairman, Regulatory Section, Animal Health Institute.)
course, are far from omnipotent, but if the job is to be done, we, individually and collectively, must accept the challenge.

The veterinary drug industry has accepted the challenge of helping to produce a safe and abundant food supply for the people of this country and the world. Each year the industry spends millions of dollars to find, test, and develop new products which will help the producers of animal food produce a better product at less cost. The cost, of course, is all important. Unless a product returns the user a benefit equal to or greater than its cost, he obviously will soon stop using the product. The cost of the product, moreover, must include all the costs of the research, development, FDA clearance, production, and distribution of the product or the drug manufacturer would soon go out of business. These economic factors, serve to place veterinary drugs in a category apart from drugs for human use in that the benefit of the drug can be expressed in precise cost-benefit terms.

This distinction was recognized in part by the Congress when it enacted the Animal Drug Amendments of 1968 establishing for animal drugs a separate section of the Federal Food, Drug, and Cosmetic Act. This legislation was sponsored by the Animal Health Institute which worked for six years to achieve its passage and was the first industry sponsored drug legislation ever enacted by Congress. In its report on the bill, the Senate Committee on Labor and Public Welfare stated —

"The American consumer, it is widely acknowledged, is the best fed in the world. He is so in very large part because the animal health industry has provided the farmer with the resources to maintain and enlarge herds and flocks of meat and milk-producing animals. Yet the farmer even today suffers enormous losses in disease, parasites, and insects, losses estimated by the Department of Agriculture at $2.8 billion per year. These losses not only reduce farm income, but, by reducing the supply of food, affect the availability of meat, poultry, eggs, and milk and increase the cost of the basic foods to the consumer.

Each delay in the clearance of safe and effective products for animal health perpetuates these losses. Every duplication of unnecessary controls adds to the ultimate cost of providing food for the consumer. Every lack of administrative coordination adds needlessly to the time required to provide the farmer with the resources he needs to feed an ever-growing population.

At the hearing held on H.R.3639 and in similar hearings held on earlier proposals, there was ample testimony that the existing provisions of the Federal Food, Drug, and Cosmetic Act, because they were designed primarily to be applicable to drugs and food additives for human consumption, created problems in the administration of the act with respect to animal health products. Some of the delay and lack of coordination has been cured administratively by the Food and Drug Administration. On the basis of hearings, however, it seems quite apparent that this coordination should be fostered legislatively by consolidating into one section of the act, in a coordinated fashion, under a simplified procedure, the various provisions and requirements applicable to animal-health products."

Under the very able leadership of Dr. Van Houweling, continuing progress has been made in reducing these administrative problems. There is, however, still room for improvement in the procedures for getting safe and effective drugs into the hands
of the producers of animal food products. Three means by which further improvements could be made are as follows:

1. **The consolidation within the Bureau of Veterinary Medicine of all the administrative and scientific functions needed for the complete review and approval of applications concerning new animal drugs.** As stated earlier, one of the primary objectives of the Animal Drug Amendments was to foster legislatively the administrative coordination of the handling of new animal drug applications or NADA's. It would seem logical, therefore, to carry out the full intent of Congress by bringing all these functions into the Bureau of Veterinary Medicine and giving the director of that bureau the authority to approve or deny all NADA's. The current splitting of review responsibility between several divisions of the Bureau of Science, the Office of Certification Services, and the Bureau of Veterinary Medicine only perpetuates the administrative complexities which the Congress intended to remedy with the passage of the Animal Drug Amendments of 1968. This diffusion of responsibility also makes it more difficult for industry to ascertain and thereby comply with the FDA's requirements for the data needed for approval of a NADA.

2. **The elimination of the Form FD-1800 or the medicated feed application.** The draft report of the FDA Study Group — popularly known as the “Kinslow Report” — commented as follows concerning these applications:

   "The ultimate impact of the medicated feed preclearance procedure is at best indirect with respect to human health protection. The necessary paperwork involved in preclearing each new drug and mixtures of these drugs in finished feeds is approaching the point where it will be insurmountable. Since the vast majority of veterinary drugs from a quantitative point of view are marketed through medicated feeds, some registration and control procedure of these individual products is needed for adequate surveillance and compliance activities. While the study group does not have a concrete recommendation, it cannot escape the view that there must be some method by which we could avoid the paper mountain created by medicated feed applications and still maintain equivalent consumer protection."

   The Animal Health Institute has endorsed this view and on August 20 directed a letter to Commissioner Ley proposing a statement of policy which would maintain adequate surveillance and control of medicated feeds while freeing the feed manufacturers, the drug industry and the FDA of a mountain of needless paperwork. The Commissioner, unfortunately, felt that he could not accept this proposal. It is hoped, however, that the FDA and AHI may yet be able to find some means of accomplishing this objective.

3. **The adoption of the recommendation of the FDA Study Group that the FDA give provisional approvals of new animal drug applications permitting final proof of efficacy through field use of the drug.** As suggested in the Kinslow Report, this procedure would be most readily applicable to the use of low levels of drugs in feeds for improving growth and feed efficiency where the safety of the drug is not in question. A specific example might be the use of a growth promotant in combination with a coccidiostat in broiler feeds. In the usual situation, the safety and efficacy of the growth promotant and the coccidiostat have already been proven by extensive data developed by the
Manufacturer of each drug. It would seem reasonable, therefore, to issue provisional approvals for combinations of these classes of drugs allowing efficacy of the combination to be proven on the basis of field use. Coupled with the elimination of the FD-1800, this would permit feed manufacturers to use those combinations of coccidiostats and growth promotants which they have found most effective in their own very rigorous tests and would free valuable and talented FDA scientists for more important matters.

We, of course, commend Dr. Van Houweling and his staff on the current rapid processing of FD-1800's as recently reported in the trade press. This situation, however, seems at least partly due to the fact that there have been very few clearances of new feed additives and combinations over the last few months.

As stated in the report of the Senate Labor and Public Welfare Committee, the animal health industry has made significant contributions to the production of an abundant, nutritious, and wholesome food supply for the American consumer. The industry has accepted the challenge of continuing these contributions in the future. In doing so, however, it recognizes its responsibility to addures at all times that the safety of animal food products for the consumer must not be jeopardized. It is our hope that we can continue to work constructively and cooperatively with the FDA, and perhaps with groups like the U.S. Animal Health Association, to meet this challenge.
The Pharmaceuticals Committee recognized the continuing problem of drug residues in tissues of animals given drugs to increase growth rate and feed utilization, and for treatment and control of disease. In connection with this problem, the committee knows of no evidence that drug residues attributable to pharmaceutical agents, such as streptomycin, neomycin, and sulfonamides, have been detrimental to human health when ingested by man in food products in the form of residues.

Further, the committee has found no convincing evidence that resistance to drugs has developed in organisms pathogenic to man as a result of the use of these drugs in animals. Development of resistance does not appear to be the potential hazard it was once thought to be, since long term use of parenteral administration and udder infusion is not commonly practiced in food producing animals. The statement attributed to Dr. R. Radeleff (Texas A & M University), “Frank poisoning by chemicals in food of animal origin has not been observed,” is still valid.

There is substantial evidence to show that antibacterial agents have been valuable in disease control, economical meat production, and in improvement of meat quality. Without these agents animal food products would not have been produced as abundantly or as economically as they now are.

The Pharmaceuticals Committee recommends that those responsible for promulgating rules and regulations with respect to use of drugs in animals not deemphasize or overlook the benefits derived from the use of these drugs because of overemphasis of the potential hazards and risks; and, further, that those responsible interpret the provisions of the law and apply them on the basis of concrete evidence of harm rather than on the basis of theoretical or speculative hazards.
INTRODUCTION

The livestock and poultry industries of the United States produced cash farm receipts of approximately $25.5 billion in 1968. These industries provide food and fiber used by nearly all Americans and employment for many. The United States is the largest exporter of farm products which account for about 20 percent of world agricultural trade.

In order for us to sell our agricultural products in world trade, we must buy agricultural products from many countries around the world. Precautions are taken to prevent the introduction of a ravaging or debilitating livestock or poultry disease into the United States via such products. For example, live domestic animals can be imported into the country only after prior approval is obtained and requirements are met regarding the disease history of the country of origin and specified tests are applied. In addition, livestock and poultry which are accepted must pass testing and/or quarantine requirements after arrival in the United States. Live poultry may be imported into the United States from most every country in the world but are subjected to a 21 day quarantine after arrival in the United States. In spite of these precautions, diseases of animals, not previously reported, do appear in the United States.

Duck virus enteritis (DVE)* or duck plague of ducks, geese, and swans has been reported as duck plague in the Netherlands and was first described as a separate virus disease there in 1942.¹ The disease was also confirmed in India in 1963 and in Belgium in 1964.¹⁶,⁶ DVE was suspected to be present in France in

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*Duck virus enteritis (DVE) and duck plague are the same diseases as described by Jansen (1949)¹⁰ and by Leibovitz and Hwang (1968).¹⁴ The duck virus enteritis term was selected for this condition because it was technically more descriptive than the term “duck plague”.

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1949 and in China in 1964.\textsuperscript{15,11} The infection was first found on the American continent in 1967 as reported by Leibovitz and Hwang (1968).\textsuperscript{14} It is the purpose of this paper to present a status report on DVE in waterfowl in the United States and describe steps taken and progress being made by State and Federal agencies and industry groups to control and eliminate the disease. Considerable attention was given to DVE in the Report of the Committee on Transmissible Diseases of Poultry (see Proceedings, USLSA, October 1968). Also included in this paper is a list of Selected References on DVE and the procedures followed in the clean-up program for DVE in White Pekin ducks as provided in the Code of Federal Regulations, Title 9, Part 83 (see Appendix).

**PART I. HOST RANGE OF DUCK VIRUS ENTERITIS (DVE)**

Only birds belonging to the family Anatidae (ducks, geese, and swans) are known to be susceptible to DVE, except day-old chicks have been experimentally infected with the DVE virus.\textsuperscript{12} Based on experiments in the Netherlands, the adult chicken hen is not susceptible to DVE. After 15 passages in day-old chicks the virus was still virulent for ducks and sometimes also for chicks up to two weeks old. Adult pigeons are not susceptible, nor were rabbits, guinea pigs, rats, or mice.\textsuperscript{8,9} Susceptibility test results conducted at the Plum Island Animal Disease Laboratory in 1968 revealed that certain species of waterfowl are susceptible to DVE while others are not (see Fig. 1).\textsuperscript{5}

**PART II. CHARACTERISTICS OF THE AGENT**

The agent of DVE has been characterized by Dardiri et al., (1967) as follows:\textsuperscript{5}

(a) The virus is not hemagglutinating or hemadsorbing.

(b) After modification by cell culture passage, it will not produce overt disease signs, but will protect susceptible ducks against virulent virus.

(c) The virus has been transmitted intramuscularly, orally, intranasally, by intracloacal swabbing, and also by direct and indirect exposure. The virus was isolated by cloacal and esophageal swabbing 45 days after artificial inoculation into susceptible ducks.

(d) The virus was inactivated in 10 minutes at 56\textdegree C or 60\textdegree C and after 2 hours at 50\textdegree C. At 22\textdegree C, it decayed slowly, with total loss of infectivity after 30 days and in 9 days when dried over calcium chloride. The virus was stable at pH 5-9, but inactivated instantaneously at pH 3 and 11.

(e) Electron microscopy of samples taken during virus development showed formation of characteristic nuclear particles. Enzymic digestion of thin sections showed both modified live virus from the Netherlands and Long Island duck enteritis virus were DNA viruses and identical. The most likely classification would place the agent in the herpesvirus group.

**PART III. SIGNS AND GROSS PATHOLOGY**

The signs and gross pathology of DVE was reported by Leibovitz and Hwang (1968) as follows:\textsuperscript{14}

**General** — The disease in the individual affected duck in the United States is acute or peracute. The disease is self-limiting within an exposed population. The
**Ducks:**
- Pekin
- Mallard
- Scaup
- **CANADA GEESE**
- **MUTE SWANS**

**Non-susceptible**
- **HERRING GULLS**
- **COWBIRDS**

DVE also known as Duck Plague
Conducted at Plum Island Animal Disease Laboratory, N.Y.

Fig. 1 — Ducks, geese, and swans (family Anatidae) are the only waterfowl known to be susceptible to DVE. Even though a limited number of species were tested, all ducks, geese, and swans must be considered susceptible to DVE.

Significant symptom was sudden death. Sick ducks that remained alive showed signs of weakness, ataxia, soiled vents, and a watery diarrhea, with very little fecal substance. Affected ducks were listless and unable to stand, and maintained a posture with wings outstretched and head down, indicating weakness and depression. The affected duck usually showed early liver lesions of petechial hemorrhages and pinpoint white spots on a pale, copper-colored friable liver. Certain distinctive pathologic features related to age were noted:

**Young Breeding Ducks** — Marked petechial and ecchymotic hemorrhages could be found widely distributed over the abdominal and thoracic organs. Esophageal and cloacal lesions were pronounced.

**Mature Breeding Ducks** — Mature breeder ducks in all of the outbreaks reported had a 25-40 percent drop in egg production. (Outbreaks in young breeders have not resulted in a severe drop in production.) Pens with the greatest percentage of mortality, the period of lowest egg production coincided with the time of greatest mortality (see Fig. 2 and Fig. 3).

There is a heavier mortality in flocks under stress of egg production as compared to immature breeders (see Fig. 4).[^1]
EGG PRODUCTION without DVE INFECTION

EGGS

400
300
200
100
0

Dec. 1966

FLOCK OF 400 HENS AND 58 DRAKES IN FULL PRODUCTION (2-YEAR OLD)
DUCK VIRUS ENTERITIS ALSO KNOWN AS DUCK PLAGUE

FLOCK MORTALITY

0 25 30 5 10 15 20 25 30

Egg Production

Flock Mortality

Jan. 1967

FIGURE 2
Fig. 3 — This flock was in approximately same stage of production as flock in Fig. 2 which did not become infected. Note high mortality and low egg production as compared to Fig. 2.
STATUS OF DUCK VIRUS ENTERITIS

DVE MORTALITY

MATURE

IMMATURE

FARM "A" 1,355 2-YEAR OLD DUCKS IN FULL EGG PRODUCTION
FARM "B" 917 DUCKS UNDER 5-MONTHS OF AGE

NOTE DUCK VIRUS ENTERITIS ALSO KNOWN AS DUCK PLAGUE

U.S. DEPARTMENT OF AGRICULTURE ANIMAL HEALTH DIVISION AGRICULTURAL RESEARCH SERVICE

Fig. 4 — Stress from egg production influences the severity of mortality losses in infected flocks.

PART IV. CONTROL AND ERADICATION MEASURES

The first outbreak involving four farms under one management was reported early in January 1967 in Suffolk County, Long Island, N.Y.¹⁴. This is the area widely known for producing the "Long Island Duckling" (see Fig. 5).

When the disease was confirmed, based on tests conducted both at the Long Island Duck Research Laboratory (LIDRL) and the Plum Island Animal Disease Laboratory (PIADL), a stamp-out program was begun.* The program was carried out cooperatively between the industry's Long Island Duck Cooperative, the New York State Department of Agriculture and Markets, and the Animal Health Division, ARS, USDA.

All White Pekin ducks and other waterfowl on the four farms of the first outbreak were euthanized with carbon monoxide gas and buried along with all eggs produced by these birds. This involved the destruction and burial of 7,067 ducks and 76,361 eggs. Then the houses, pens, and equipment were thoroughly cleaned and disinfected. Litter from the houses was burned. Federal and State indemnity funds were paid the flock owner.²⁰ All other commercial White Pekin duck flocks

*Laboratory support of program efforts was ably provided by the Cornell University Duck Research Laboratory, Eastport, L.I., N.Y., and the Plum Island Animal Disease Laboratory, Greenport, L.I., N.Y., a facility of the Animal Disease and Parasite Research Division, ARS, USDA.
in the County were placed under close surveillance and blood samples were collected and tested from 13 randomly selected flocks to determine if there was evidence of experience with the DVE agent.

Flocks on three farms (in addition to the four farms involved in the first outbreak) yielded serum which contained DVE virus neutralizing antibodies based on test standards. These flocks never have shown clinical signs of the disease.

Fig. 5 — The shaded areas indicate intensive-type White Pekin duck producing areas which consist of 34 operating units.

In mid-March 1967, lesions of DVE appeared in ducks from two flocks that were neighbors to the first outbreak farms. Collection of blood samples from domestic, captive, and migratory waterfowl in several States was begun. Test results were negative for all samples from outside New York. This was evidence that the disease had not yet spread to other parts of the United States, and action should be taken to prevent this.

On May 9, 1967, Suffolk County was placed under Federal quarantine in an effort to prevent the interstate spread of the disease through the movement of domestic waterfowl and their products outside of the infected area. Prior to this, individually infected flocks were placed under State quarantine to prevent spread of the disease within the quarantined area through the movement of ducks or their
products.

During 1967 a total of 15 White Pekin duck flocks of the 41 flocks in operation at that time were determined to be infected sometime during the year. A second collection of blood samples was conducted in August 1967 from young replacement White Pekin breeder flocks in Suffolk County. Twenty-one of 22 tested negative. This gave encouragement to the initiation of a program designed around isolating and controlling the disease in known infected flocks followed by slaughter of the infected flock as soon as feasible. Replacement breeders were reared separately. Control measures were instituted which included the following: (a) cooking of slaughter waste from infected flocks and flocks of unknown DVE status; (b) prompt disposal by rendering of dead waterfowl from all causes from all flocks; (c) control of movements of farm personnel, equipment, and service people between farms; (d) prompt investigation of reported outbreaks and issuance of farm quarantines when new infection was disclosed; (e) utilization of cleaning and disinfecting equipment on farm facilities between groups of growing and breeding ducks; and, finally, beginning in early 1968, (f) the application of a modified DVE live vaccine to all ducks on known infected premises and 50 percent of the 100,000 breeder ducks. An outline of procedures (CFR, Title 9, Part 83) for approving domestic waterfowl flocks and other pertinent data is given in the Appendix.

Extensive epidemiological investigation on the possible avenues of entry and spread of DVE in the United States gave further support and substance to the measures in the program put into operation.

During 1968, after sustained application of the above program, 4 of the 15 outbreak farms of 1967 continued to have intermittent to extensive clinical signs of the disease and one new flock became infected (see Fig. 6). The program had worked on the other 11 farms. The last signs of DVE in White Pekin flocks were seen in November 1968. By August 1969 all flocks were shown by surveillance and testing procedures to be free of infection and were declared “approved” as provided for in the Federal quarantine.

On September 25, 1969, the Federal quarantine of Suffolk County domestic waterfowl because of DVE was released. A six-months surveillance is being maintained by mutual agreement between the Long Island duck industry and the State and Federal disease control officials.

PART V. IMPACT OF THE DISEASE ON THE WHITE PEKIN DUCK INDUSTRY AND OTHER WATERFOWL POPULATIONS

White Pekin Ducks — When DVE first appeared there were 41 duck farms in Suffolk County, L.I., N.Y. Today there are 34 duck farms producing approximately the same number of commercial ducks. The greatest impact of the disease was the losses sustained while the disease was clinically evident. One farmer discontinued business permanently because of DVE and, in addition, some reduced operations or discontinued business temporarily. In 1967 losses to the Long Island duck industry because of DVE were conservatively estimated to exceed $400,000 due to mortality and reduced production.

Captive Waterfowl — The U.S. Department of the Interior (USDI) issued over 4400 permits (licenses) in 1968 to persons for the rearing, selling, or research on captive waterfowl; these persons are frequently referred to as aviculturists. This list
DUCK VIRUS ENTERITIS
Outbreaks Diagnosed in Waterfowl Flocks

**Commercial**

- 15 cases in 1967
- 5 cases in 1968
- 5 cases in 1969

**Captive**

- 3 cases in 1967
- 2 cases in 1968
- 3 cases in 1969

**Migratory**

- 12 cases in 1967
- 1 case in 1968
- 1 case in 1969

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Fig. 6 — Outbreaks of DVE in commercial waterfowl frequently involved several age groups on one farm. Outbreaks in captive waterfowl were widely dispersed on farms located in three States in the migratory waterfowl Atlantic flyway.
STATUS OF DUCK VIRUS ENTERITIS

Fig. 7 — Thirty-four White Pekin duck flocks, representing over 100,000 breeders and yearly production of approximately 5½ million market ducks, and two aviculturists, owning many species of captive waterfowl, became eligible to be classified as “approved” (not infected) at the rate shown under U.S. Department of Agriculture and Markets, and Cornell University Duck Research Laboratory supervision.

is not considered complete for all persons who rear captive waterfowl (see Fig. 8).

The disease was diagnosed in captive waterfowl on a premises in Suffolk County, N.Y., and also in a similar type flock on a premises 200 air miles northwest of Suffolk County in Chemung County in upstate New York.* No waterfowl shipment relationship between the two outbreaks was determined. In December 1967 the virus of DVE was isolated again from a mallard duck found dead on the Suffolk County captive waterfowl premises that had been infected in May 1967.13

In June 1968 the agent of the disease was recovered from a black duck found dead in a Maryland captive waterfowl flock about 200 miles southwest of the first outbreak area in Suffolk County. The disease appeared next and the DVE agent was

*Diagnosis of 37 outbreaks of DVE in the U.S. is based on viral agent isolation with demonstrated ability to neutralize DVE antiserum; or a combination of both gross pathological lesions considered indicative of the disease and detection of DVE antibodies in blood serums from the flock. Such blood serums should neutralize either the field strain or modified strain of the agent. Outbreaks are identified as distinct, separate, but not necessarily unrelated expressions of the disease.
recovered from an Egyptian and Canada geese flock in Bucks County, Pa., about 75 miles from Suffolk County and midway between Suffolk County, N.Y., and the outbreak in the State of Maryland.

The disease reappeared in May 1969 (last known outbreak of the disease in the United States) when outbreaks were diagnosed in mallards on a shooting club near the Hudson River in Duchess County, N.Y., about 50 miles northwest of Suffolk County, N.Y., and in two widely separated small Muscovy duck flocks in Dauphin and Montgomery Counties, Pa. No relationship could be established between these 3 outbreaks. One common element was exposure to visiting migratory waterfowl.

Fig. 8 — Note the calendar order of the countries in which DVE appeared. Types of waterfowl affected according to outbreak were:
Outbreak No. 1 — White Pekin ducks, captive waterfowl, migratory waterfowl;
Outbreak No. 2, 4, 5, 6, 7, 8 — captive waterfowl only;
Outbreak No. 3 — migratory waterfowl only.

The impact of DVE on U.S. captive waterfowl can be severe in those flocks affected and financial loss can be high, particularly in instances involving rare or valuable species. DVE does not appear to be a disease threatening to destroy huge parts of the captive waterfowl populations.

Migratory or Free Flying Waterfowl — The first case of DVE in free flying waterfowl was in a mute swan found dead February 1, 1967, on the first infected White Pekin duck premises. This was 28 days after the infection was diagnosed in
STATUS OF DUCK VIRUS ENTERITIS

White Pekin ducks. Twelve of 13 field outbreaks of DVE in migratory waterfowl occurred in association with the infection among White Pekin ducks in Suffolk County, N.Y. (see Fig. 6). The largest number of migratory waterfowl found dead in association with these outbreaks was in the Flanders Bay area in Suffolk County, N.Y., occurring at or soon after the peak of infection in White Pekin ducks in November and December 1967. The last known outbreak in migratory waterfowl occurred in May 1968 near Oakdale, N.Y., in Suffolk County (Long Island).

As has been mentioned, in 1968 in order to determine if DVE was established in migratory and commercial waterfowl in other parts of the country, over 3,000 blood samples were collected at 14 locations in 12 States in the northeastern and southeastern United States. Tests conducted on the blood revealed no samples with neutralizing antibodies at a level considered to be positive. In addition, die-offs which occurred among migratory waterfowl in Delaware, Florida, Maryland, Massachusetts, Pennsylvania, and Virginia were investigated to determine if DVE was involved. DVE was not involved in any of these. It was concluded that DVE was not established in the populations of tested waterfowl, or if present it was at levels not possible to detect by the procedures utilized. Surveillance via serological testing is very time consuming and expensive but it was considered essential that attempts be continued to look for the disease.

Therefore, the DVE surveillance program involving migratory and other waterfowl is conducted by providing DVE test antisera to disease diagnostic laboratories that normally receive specimens from such birds. These laboratories, located in 7 States and scattered throughout the migratory waterfowl flyways are cooperating with the USDI and USDA in maintaining surveillance to determine if, and to what extent DVE may still exist in American waterfowl (see Fig. 9).

Evidence suggests the following conclusions about DVE in migratory waterfowl:

(a) Outbreaks in migratory waterfowl could have occurred because of exposure to DVE from infected White Pekin duck flocks. However, the original outbreak in White Pekin ducks could have been introduced by free flying waterfowl that contracted the disease from other types of carrier imported waterfowl.

(b) The disease is causing little or no on-going problems in migratory waterfowl, but it bears watching.

(c) Circumstantial evidence exists to support the thesis that migratory waterfowl could have served as carriers of the disease to captive waterfowl. Artificially infected birds shed the virus up to 45 days post inoculation.

(d) Migratory waterfowl may yet serve as a vehicle to spread DVE among themselves and to commercial and captive waterfowl in other parts of the country.

(e) If the disease does not spread to other flyways within 4 years of the last known outbreak, there is a high probability it will not occur because there is a 50 percent natural replacement of migratory waterfowl each year.

(f) Since the DVE agent is a herpestype virus, it may have the ability to go into an extended eclipse phase in which the agent could not be detected and may create a situation conducive to the establishment of numerous carrier birds.

(g) On the other hand, since migratory waterfowl are free flying and not confined there is less opportunity for exposure to sufficient dosage of virus to produce
clinical disease as compared to closely confined Pekin ducks. This has the effect of diluting the disease.

Fig. 9 — DVE monitoring program is carried out cooperatively between USDA, USDI, State animal disease control agencies, State wildlife agencies, and several State universities. The program is designed to test wildlife specimens for DVE when submitted to laboratories in States indicated above. Confirmation testing is conducted at USDA’s Plum Island Animal Disease Laboratory.
Fig. 10 — These maps illustrate the pattern of migratory waterfowl movements which provide a possible means for spreading DVE to captive waterfowl, commercial waterfowl, and other migratory waterfowl. Only close surveillance and monitoring of susceptible populations for evidence of the disease will disclose whether DVE exists in reservoir in migratory waterfowl.

Atlantic Waterfowl Flyway — From Waterfowl Tomorrow, U.S. Department of the Interior, 1964
Fig. 10 — Continued

MISSISSIPPI WATERFOWL FLYWAY

From Waterfowl Tomorrow, United States Department of the Interior, 1964
Fig. 10 – Continued

CENTRAL WATERFOWL FLYWAY

From Waterfowl Tomorrow, United States Department of the Interior, 1964
Fig. 10 — Continued

PACIFIC WATERFOWL FLYWAYS

From Waterfowl Tomorrow, United States Department of the Interior, 1964
SUMMARY

Outbreaks of DVE were diagnosed in Anatidae in 8 counties in three States in the United States during 1967, 1968, and 1969 (see Fig. 8 and Fig. 11). After being diagnosed in White Pekin ducks in Suffolk County, L.I., N.Y., in January 1967, the program described in this paper was carried out for 33 months. Infection in the Pekin duck industry was last seen November 1968. All outbreaks of DVE were placed under Federal or State quarantine, or both, to prevent both interstate and intrastate movements of infected flocks.

The disease was diagnosed in 37 outbreaks in 6 species of ducks, 2 species of geese, and in mute swans. Each outbreak was handled individually except for those in Suffolk County where 41 farms were handled as a total industry. Sixteen outbreaks were in commercial ducks, 8 outbreaks in captive waterfowl, and 13 outbreaks were in migratory waterfowl.

Depopulation of infected flocks is the preferred procedure in dealing with outbreaks of DVE; however, experience shows that keeping an outbreak flock confined, applying sound disease prevention and eradication management practices, cleaning and disinfecting of equipment and premises, and judicious use of modified live virus vaccine provide a means for control and eventual cleanup of infected flocks. This thesis is yet to be proven entirely; however, based on experiences described in this paper to date, these procedures were successfully applied in the
White Pekin duck industry.

Federal quarantine restrictions on the interstate movement of domestic waterfowl and their products from Suffolk County, L.I., N.Y., were removed September 25, 1969. Surveillance will continue for an additional six-months period in the White Pekin duck industry.

The spread of DVE from duck farm to duck farm in Suffolk County was aided by rearing practices of the industry and also migratory waterfowl.

Surveillance of captive and migratory waterfowl populations through cooperative efforts between local and Federal Government agencies will continue for an extended period of time in an attempt to obtain the needed information on the possible subclinical reservoirs of DVE in susceptible species.

All future outbreaks of DVE should be handled as an exotic disease, applying measures aimed at eradication.

The last known outbreak of the disease in the United States was in captive mallards in May 1969 in upstate New York.

Captive waterfowl in the United States are apparent victims of DVE from Pekin ducks transmitted by migratory waterfowl.

The specific means of introduction of DVE into the United States is not known. Introduction via birds flying over from Europe or Asia, or exposure to North American waterfowl to European or Asian waterfowl in Canadian nesting areas cannot be ruled out entirely. It is possible that DVE was present in free flying waterfowl prior to 1967 and not recognized. However, evidence suggests most strongly that nonclinical carrier waterfowl importations from Europe (domestic, captive, or released free flying waterfowl) would have to be rated high as a probably source. Consideration should be given to establishing additional import requirements, especially subjecting imported waterfowl to testing prior to entry and/or while in quarantine in the United States.
APPENDIX

Title 9—ANIMALS AND ANIMAL PRODUCTS

Chapter I—Agricultural Research Service, Department of Agriculture

SUBCHAPTER C—INTERSTATE TRANSPORTATION OF ANIMALS AND POULTRY

PART 83—DUCK VIRUS ENTERITIS (DUCK PLAGUE)

Sec.
83.1 Definitions.

83.2 Notice relating to existence of the contagion of duck virus enteritis and notice of quarantine.

83.3 Notice of regulations.

83.4 Interstate movement of affected or exposed domestic waterfowl and products thereof prohibited.

83.5 Interstate movement of domestic waterfowl, products thereof, and related articles from quarantined areas restricted.

83.6 Other movements.

83.7 Approval and maintenance of source flocks.

83.8 Approval of hatcheries.

83.9 Termination of approval of flocks and hatcheries.

83.10 Cleaning and disinfection.


Source: The provisions of this Part 83 appear at 33 P.R. 7013, May 9, 1967, unless otherwise noted.

§ 83.1 Definitions.

As used in this part, the following terms shall have the meanings set forth in this section.

(a) "Department" means the U.S. Department of Agriculture.

(b) "Division" means the Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture.

(c) "Director of Division" means the Director of the Animal Health Division, or any other official of the Division to whom authority has heretofore been delegated or may hereafter be delegated to act in his stead.


(e) State Inspector: An inspector regularly employed in livestock sanitary work of a State or political subdivision thereof, and who is authorized by such State or political subdivision to perform the functions involved.

(f) Accredited Veterinarian: A veterinarian approved by the U.S. Department of Agriculture to perform the functions involved.

(g) State: Any State, Territory, the District of Columbia or Puerto Rico.

(h) Interstate: From one State into or through any other State.

(i) Quarantined area: A State or area quarantined under this part because of duck virus enteritis.

(j) Nonquarantined area: Any State or area not quarantined under this part because of duck virus enteritis.

(k) Domestic waterfowl: Ducks, geese, swans, and other waterfowl of all ages in captivity.

(l) Duckling: An unfed newly hatched duck.

(m) Domestic waterfowl products: Eggs, feathers, manure, carcasses, parts thereof, or offal derived from domestic waterfowl in a quarantined area.

(n) Duck virus enteritis: The contagious, infectious, and communicable disease of waterfowl, also known as duck plague.

(o) Approved source flock: A flock of domestic waterfowl approved in accordance with § 83.7.1

(p) Approved hatchery: A domestic waterfowl hatchery approved in accordance with § 83.8.

(q) Nonapproved flock: A flock of domestic waterfowl in a quarantined area which does not meet the requirements of § 83.7.

(r) Approved laboratory: A laboratory approved by the Director of Division for the diagnosis of duck virus enteritis.

(s) Feed bags: Burlap, canvas, paper or other bags which have been used to contain any livestock or poultry feed on any premises where domestic waterfowl were maintained in a quarantined area.

(t) Approved vaccine. A vaccine for the control of duck virus enteritis which is produced and tested in accordance with the regulations in Subchapter E of this chapter. A list of approved vaccines may be obtained from the Director of the Veterinary Biologics Division.

1 Names of approved domestic waterfowl source flocks and approved hatcheries may be obtained from the Director, Animal Health Division, Agricultural Research Service, Hyattsville, Md. 20782, upon request.
§ 83.2 Notice relating to existence of the contagion of duck virus enteritis and notice of quarantine.

Notice is hereby given that the contagion of duck virus enteritis exists in Suffolk County in the State of New York and that live poultry in that county are affected with said disease. Therefore, Suffolk County is hereby quarantined.

§ 83.3 Notice of regulations.

Notice is hereby given that in order to more effectually suppress and extirpate duck virus enteritis, to prevent the spread thereof, and to protect the domestic waterfowl industry of the United States, the regulations in this part are promulgated.

§ 83.4 Interstate movement of affected or exposed domestic waterfowl and products thereof prohibited.

Domestic waterfowl affected with or exposed to duck virus enteritis and domestic waterfowl products derived from such affected or exposed waterfowl may not be moved interstate for any purpose. A bird shall be deemed to be exposed to duck virus enteritis within the meaning of this part if it was in a flock in which any bird was affected with the disease or if it was otherwise in contact with an affected bird within the preceding 10 days.

§ 83.5 Interstate movement of domestic waterfowl, products thereof, and related articles from quarantined area restricted.

Domestic waterfowl, domestic waterfowl products, and feed bags shall not be moved interstate from any quarantined area except in accordance with the provisions of this section. Authorization for such movement under such provisions does not excuse compliance with other applicable Federal or State laws or § 83.4.

(a) Newly hatched domestic waterfowl. Ducklings or newly hatched domestic waterfowl of other species may be moved interstate from a quarantined area if they originate in approved source flocks, were hatched in approved hatcheries, and are certified by a State or Federal inspector as meeting these requirements.

(b) Other domestic waterfowl. Domestic waterfowl, other than newly hatched domestic waterfowl, may be moved interstate from a quarantined area if they (1) originate from an approved source flock; (2) were hatched in an approved hatchery or under natural conditions on the farm of origin; (3) have been tested for duck virus enteritis by a State or Federal inspector and found to be negative; and (4) are not known to have been exposed to duck virus enteritis and are certified by a State or Federal inspector as meeting these requirements.

(c) Carcasses and parts of domestic waterfowl. Eviscerated carcasses of domestic waterfowl processed, inspected and passed at federally inspected slaughtering establishments located within a quarantined area may be moved interstate without further restriction under this part.

(d) Offal; feathers; manure; carcasses and parts of carcasses condemned because of duck virus enteritis. Offal, feathers, and manure, and carcasses and parts of carcasses condemned because of duck virus enteritis, which are derived from nonapproved flocks of domestic waterfowl in a quarantined area may be moved interstate under this part if they have been heated throughout to a temperature of not less than 140° F. for not less than 10 minutes, or have been heated according to a method specifically approved by the Director of Division. Offal, feathers, and manure derived from approved source flocks may be moved interstate without restriction under this part.

(e) Feed bags. Feed bags used on premises where nonapproved flocks of domestic waterfowl are maintained may be moved interstate from a quarantined area if they have been heated throughout to a temperature of not less than 140° F. for not less than 10 minutes, or heated according to a method specifically approved by the Director of Division. Other feed bags may be moved interstate without restrictions under this part.

(f) Eggs. Clean eggs from domestic waterfowl may be moved interstate from a quarantined area if they originate in approved source flocks, are not known to have been exposed to products or equipment or personnel which may have been contaminated by nonapproved source flocks, and have been sanitized by means of a final rinse which contains (1) 200 parts per million of free chlorine or (2) 400 parts per million of a quarternary ammonium cationic detergent, and are certified by a State or Federal inspector as meeting these requirements.

[39 F.R. 11899, Aug. 22, 1969]

§ 83.6 Other movements.

The Director of Division may in specific cases, authorize the interstate movement from a quarantined area of domestic waterfowl, domestic waterfowl products, or feed bags, not otherwise authorized under this part, under such conditions as he may prescribe to prevent the spread of duck virus enteritis: Provided, That they
shall be accompanied by a permit from the appropriate official of the State of destination.

§ 83.7 Approval and maintenance of source flocks.

(a) A breeding flock or commercial flock of domestic waterfowl may be designated an approved source flock by the Director of Division after a period of 60 days during which the flock has been under the supervision of a Federal or State inspector, if:

1) Flock records including mortality and egg production, when appropriate, are available for inspection by State or Federal inspectors;

2) Any waterfowl that die in the flock are submitted to an approved laboratory for examination as requested by a State or Federal inspector and do not show pathological lesions of duck virus enteritis;

3) The flock is not known to have been exposed to duck virus enteritis.

4) The flock meets one of the following requirements:

(i) No evidence of duck virus enteritis has ever been known to exist in domestic waterfowl located on the premises; or

(ii) No evidence of duck virus enteritis has existed on the premises during the past 12 months and the flock at the time of approval is not known to have been affected with duck virus enteritis; or

(iii) The premises has been completely depopulated and thoroughly cleaned and disinfected under the supervision of a Federal or State inspector and left vacant for a period of 21 days, and such premises is repopulated with waterfowl from an approved source; or

(iv) No evidence of duck virus enteritis exists in waterfowl now located on the premises and the flock has been tested for duck virus enteritis by a State or Federal inspector and found to be negative.

5) When flocks are vaccinated against duck virus enteritis, only an approved vaccine is used, it is used under the supervision of a State or Federal inspector and it is used only in flocks not known to have been affected with the disease.

(b) Maintenance of approved status: A breeding or commercial domestic waterfowl flock which has been designated an approved source flock by the Director of Division shall retain such status so long as the applicable provisions of paragraph (a) of this section are complied with under the supervision of a State or Federal inspector.

§ 83.8 Approval of hatcheries.

A domestic waterfowl hatchery may be designated an approved hatchery by the Director of Division if:

(a) The hatchery is maintained and operated in accordance with requirements specified in 9 CFR 147.33, and in such manner as to prevent the transmission of duck virus enteritis under the surveillance of a State or Federal inspector.

(b) Records identifying all eggs hatched as to source, fertility, and hatchability, and records of all shipments from the hatchery are maintained, and such records are made available for inspection by State or Federal inspectors as deemed necessary by the Director of Division.

(c) Only eggs from approved source flocks are hatched on the premises. [32 F.R. 7012, May 9, 1967, as amended at 32 F.R. 6079, June 6, 1967]

§ 83.9 Termination of approval of flocks and hatcheries.

Whenever it is determined by a State or Federal inspector that a domestic waterfowl flock or a hatchery which has been approved under the provisions of § 83.7 or § 83.8 no longer meets the requirements of such provisions for such approval, he shall terminate such approval by giving notice thereof to the owner of the flock or hatchery involved. Prior to making any such determination, he shall inform the owner of his proposed action and the reasons therefor and afford the owner opportunity to present his views with respect to the proposed action.

§ 83.10 Cleaning and disinfection.

Cars, trucks, and other vehicles, coops, containers, and other facilities used in connection with the interstate shipment of domestic waterfowl affected with or exposed to duck virus enteritis or domestic waterfowl products derived from such affected or exposed waterfowl shall be cleaned and disinfected in accordance with provisions of §§ 71.4-71.11 of this subchapter, or with a 3-percent solution of cresol compound U.S.P.
PART 83—DUCK VIRUS ENTERITIS
(DUCK PLAGUE)

Areas Quarantined

§ 83.2 Notice relating to existence of the contagion of duck virus enteritis and notice of quarantine.

(a) Notice is hereby given that the contagion of duck virus enteritis exists in Suffolk County in the State of New York and that live poultry in that county are affected with said disease. Therefore, Suffolk County is hereby quarantined.

(b) Further notice is hereby given that the contagion of duck virus enteritis exists on the premises of the Patuxent Wildlife Research Center, U.S. Department of the Interior, located at Laurel, Md., in Prince George's and Anne Arundel Counties of the State of Maryland, and that live poultry on such premises are affected with said disease. Therefore, the premises of the Patuxent Wildlife Research Center, Laurel, Md., are hereby quarantined.

Effective date. The foregoing amendment shall become effective upon publication in the Federal Register.

The purpose of this amendment is to add the premises of the Patuxent Wildlife Research Center, Laurel, Md., to the list of areas quarantined because of the contagion of duck virus enteritis (duck plague); to facilitate the confinement of said disease to areas where the contagion is known to exist; and to prevent the spread of duck virus enteritis from affected flocks in the quarantined area.

The amendment imposes certain restrictions necessary to prevent the spread of duck virus enteritis, a communicable disease of waterfowl, and must be made effective immediately in order to accomplish its desired purpose. Accordingly, under the administrative provisions of 5 U.S.C. 553, it is found upon good cause that notice and other public procedure with respect to the amendment are impracticable and contrary to the public interest, and good cause is found for making it effective less than 30 days after publication in the Federal Register.

Done at Washington, D.C., this 28th day of July 1969.

GEORGE W. IRVING, JR.,
Administrator,
Agricultural Research Service.

FR 12561 (8/1/69)
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Book


Survey

PASTEURÉLLA MULTOCIDA:
IMMUNE RESPONSE IN CHICKS AND MICE

K. L. Hedleston and P. A. Rebers

Animal Disease and Parasite Research Division,
Agricultural Research Service
United States Department of Agriculture
National Animal Disease Laboratory
Ames, Iowa 50010

SUMMARY

Comparisons were made of the immune response in chicks and mice to aqueous and emulsified fowl cholera vaccines prepared from capsulated and noncapsulated cells of killed Pasteurella multocida. Two doses of aqueous vaccine administered 10 and 17 days apart or 1 dose of emulsified vaccine were used. The vaccines were injected intraperitoneally (i.p.), subcutaneously (s.c.), and intramuscularly.

In chicks, a high degree of immunity was stimulated with both vaccines containing either capsulated or noncapsulated cells, regardless of the route of injection.

In mice, however, the vaccines prepared from capsulated cells were much more effective than those prepared from noncapsulated cells when injected i.p. The only protection obtained with noncapsulated cells was with the mice that received 2 doses of aqueous vaccine administered i.p. 17 days apart. No immunity was obtained with either emulsified vaccine when injected s.c.

These experiments demonstrated that the immune response of mice to killed P. multocida was not the same as that of chicks. Therefore, the mouse is not a suitable host for testing the efficacy of fowl cholera vaccines, or for determining immunogenic differences among isolants of P. multocida for use in fowl cholera vaccines.

INTRODUCTION

Mice are sometimes used to determine the efficacy of vaccines that are prepared for use in birds and other species of animals. Recently, Ose and Muenster reported a method in which mice were used to evaluate killed P. multocida vaccines for use in large animals. This procedure would be advantageous in testing fowl cholera vaccines if the immune response of chickens and turkeys was the same as that of mice. Priestly, using mice as the immunized host, emphasized the importance of using a highly virulent capsulated strain of P. multocida for preparing the vaccine. Yaw and Kakavas showed that saline extracted antigens from capsulated cells immunized 100% of chickens and 90% of mice, whereas noncapsulated cells immunized 90% of chickens but only 50% of mice. Hedleston et al. immunized chickens with emulsified vaccines prepared from capsulated and noncapsulated P. multocida cells and from saline extracts of the cells.

This report compares the immune response of young chicks and mice to

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aqueous and emulsified fowl cholera vaccines prepared from capsulated and noncapsulated cells of *P. multocida*.

**MATERIALS AND METHODS**

**Cultures.** Capsulated, virulent cells from iridescent colonies and noncapsulated, avirulent cells from gray colonies of *P. multocida* strains of X-73F and X-73G, respectively, were used for preparing the vaccines.

Cells from the virulent strain X-73F were used as a challenge culture in determining the immune response.

**Vaccines.** A lyophilized culture was reconstituted in tryptose broth (Difco), streaked on dextrose starch agar (Difco) and incubated 24 hours at 37°C. A single colony was suspended in broth and subcultured on agar. The cells were harvested after 24 hours in 0.85% NaCl solution containing 0.3% formalin and adjusted to 10X McFarland No. 1 density. Density was adjusted with a Coleman Jr. spectrophotometer (Model 6-A); 80% transmittance at 600 µ is approximately equivalent to a McFarland No. 1 tube density. Both the capsulated and non-capsulated cells were prepared in this manner. However, the suspension of noncapsulated cells which formed chains had to be mixed vigorously with a pipette before determining its density. For the water-in-oil emulsified vaccines, 25 ml of sterile bayol F (Esso Standard Oil Co., Linden 1, New Jersey) containing 3% Arlacel A (Atlas Powder Co., Wilmington, Delaware) was added to 25 ml of the killed bacteria, and the mixture emulsified in a VirTis homogenizer (VirTis Co., Inc., Gardner, New York) for 1 minute at a setting of 75. The aqueous vaccines were prepared by adding an equal quantity of 0.85% NaCl containing 0.3% formalin to the killed bacteria. The amount of nondialyzable solids in the culture as determined by lyophilization after dialysis against distilled water was 74 µg for the noncapsulated and 86 µg for the capsulated cells per 0.1 ml dose of vaccine.

**Chicks and mice.** Six-day-old New Hampshire chicks from the National Animal Disease Laboratory flock that has been closed for 32 years, and 18- to 20-g Swiss-Webster female mice (Simonsen Laboratories, St. Paul, Minnesota) were used when indicated. No vaccines or medications were used in the parent flock of chickens.

**Vaccination.** Twelve groups of 10 chicks and 8 groups of 10 mice were inoculated with 0.1 ml of vaccine as recorded in Tables 1 and 2.

**Determination of immunity.** For challenging the immunity, the capsulated culture was grown on dextrose starch agar for 24 hours at 37°C, suspended in broth, and adjusted to 80% transmittance at 600 µ. It was diluted 10^3 and 0.1 ml was injected intramuscularly in the thigh of each chick and intraperitoneally in each mouse. The colony-forming units per dose were determined by plate count on dextrose starch agar at 24 hours. An LD_{100} was less than 10 organisms for mice and less than 100 organisms for chicks. All chicks and mice were inoculated with approximately 1000 colony-forming units. Survivors were observed for at least 3 weeks after challenge inoculation.
RESULTS

Results are summarized in Tables 1 and 2. In chicks (Table 1), a high degree of immunity was stimulated with vaccines prepared from both capsulated and noncapsulated cells, regardless of the route or schedule of administration. There was no difference in the time required to stimulate immunity, using capsulated and noncapsulated cells. With the emulsified vaccines, chicks became immune between the 7th and 14th day postvaccination.

In mice (Table 2), the aqueous and emulsified vaccines prepared from capsulated cells were much more efficient than those prepared from noncapsulated cells when injected i.p., but no protection was obtained with either of these emulsified vaccines when injected s.c. The only protection obtained with noncapsulated cells was with the mice that received two doses of aqueous vaccine administered i.p., 17 days apart.

DISCUSSION

There was a marked difference between the capsulated and non-capsulated cells in stimulating immunity in the mice but not in the chicks. The reason for this difference, which could not be determined from the results of these experiments, may be associated with capsular polysaccharide or lipopolysaccharide antigens of P. multocida.

It is interesting to note that the various routes of vaccination did not alter the immune response in the chick, but in the mouse immunity was induced by the i.p. route and not by the s.c. route. This difference in response to i.p. and s.c. routes of vaccination in mice has been observed with typhoid vaccines.

These experiments demonstrated that the immune response of mice to killed P. multocida was not the same as that of chicks. They also emphasize the necessity of comparing various time schedules and routes of inoculation when studying an immune response in mice. Results of these experiments agree with the findings of others cited in the introduction.
TABLE 1.
Vaccination record of chicks and results of immunity challenge.

<table>
<thead>
<tr>
<th>Type of vaccine&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Vaccination Route&lt;sup&gt;B&lt;/sup&gt;</th>
<th>Day&lt;sup&gt;C&lt;/sup&gt;</th>
<th>Challenge day</th>
<th>No. died out of 10</th>
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<tbody>
<tr>
<td>X-73F aqueous</td>
<td>i.m.</td>
<td>0 17</td>
<td>27</td>
<td>2</td>
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<tr>
<td>X-73F aqueous</td>
<td>i.m.</td>
<td>0 10</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>X-73G aqueous</td>
<td>i.m.</td>
<td>0 17</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>X-73G aqueous</td>
<td>i.m.</td>
<td>0 10</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>X-73F emulsified</td>
<td>i.p.</td>
<td>0</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>X-73F emulsified</td>
<td>s.c.</td>
<td>0</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>X-73G emulsified</td>
<td>i.p.</td>
<td>0</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>X-73G emulsified</td>
<td>s.c.</td>
<td>0</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>None (controls)</td>
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<td>--</td>
<td>--</td>
<td>10</td>
</tr>
<tr>
<td>X-73F emulsified</td>
<td>s.c.</td>
<td>0</td>
<td>7</td>
<td>10</td>
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<td>10</td>
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<td>0</td>
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<tr>
<td>None (controls)</td>
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<td>--</td>
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<td>10</td>
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</tbody>
</table>

<sup>A</sup>X-73F = capsulated cells, X-73G = noncapsulated cells.
<sup>B</sup>i.m. = intramuscular, i.p. = intraperitoneal, s.c. = subcutaneous.
<sup>C</sup>Day 0 = day first dose of vaccine was administered.

TABLE 2.
Vaccination record of mice and results of immunity challenge.

<table>
<thead>
<tr>
<th>Type of vaccine&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Vaccination Route&lt;sup&gt;B&lt;/sup&gt;</th>
<th>Day&lt;sup&gt;C&lt;/sup&gt;</th>
<th>Challenge day</th>
<th>No. died out of 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-73F aqueous</td>
<td>i.p.</td>
<td>0 17</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>X-73F aqueous</td>
<td>i.p.</td>
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<td>20</td>
<td>1</td>
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<tr>
<td>X-73G aqueous</td>
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<td>27</td>
<td>3</td>
</tr>
<tr>
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<td>20</td>
<td>9</td>
</tr>
<tr>
<td>X-73F emulsified</td>
<td>i.p.</td>
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<td>1</td>
</tr>
<tr>
<td>X-73F emulsified</td>
<td>s.c.</td>
<td>0</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>X-73G emulsified</td>
<td>i.p.</td>
<td>0</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>X-73G emulsified</td>
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<td>0</td>
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</tr>
<tr>
<td>None (controls)</td>
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<td>--</td>
<td>--</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>A</sup>X-73F = capsulated cells, X-73G = noncapsulated cells.
<sup>B</sup>i.p. = intraperitoneal, s.c. = subcutaneous.
<sup>C</sup>Day 0 = day first dose of vaccine was administered.
REFERENCES


REPOR OF THE SUBCOMMITTEE ON PULLORUM-TYPHOID ERADICATION

B. S. Pomeroy, D.V.M.
St. Paul, Minnesota
USAHA — 1969

At the 1968 meeting of the Committee on Transmissible Diseases of Poultry the minimum standards of the Five Phase Program for the Eradication of Pullorum Disease and Fowl Typhoid were modified.

At the annual meeting of the National Turkey Federation in January, 1969, the proposed standard procedures were discussed with the NTF Disease Control and Eradication Committee. The committee approved in principle the proposal and referred it to the NTF Executive Committee. At a meeting in February, the NFT Executive Committee approved in principle the proposed program and suggested the NTF Disease Control and Eradication Committee and representatives from Animal Health Division and Animal Husbandry Research Division, USDA develop a more specific program for turkeys. Two meetings were held and the NTF Disease Control and Eradication Committee developed a draft that was presented to the NTF Executive Board for consideration in July. Suggestions were made and the Committee has resubmitted the proposal to the NTF Executive Committee for action in October. The present proposal has Two Phases rather than the Five Phases as recommended by the committee in 1968.

Phase 1 — Eradication Phase by Area, Combination of Phases 1,2,3, of the original proposal.

Phase 2 — Free Status by Area, Phase 4 of old proposal.

In order to qualify for Phase 1, the following authorities must exist in the State.

1. Pullorum disease — Fowl typhoid are mandatory reportable diseases in the state.
2. The State has the authority to place and maintain quarantines for these diseases.
3. All reports of these diseases are investigated by authorized state authorities.
4. The State has an importation regulation requiring shipment of turkeys and hatching eggs to be from sources considered to be free of pullorum-typhoid.
5. The State has a regulation requiring that turkeys going to exhibition come from flocks free from pullorum-typhoid.
6. 100% of the eligible turkey flocks and hatcheries in the State participate either in the NTIP or an equivalent state program.

A survey was made of the 50 states, Puerto Rico and Virgin Islands to ascertain the status of each state as it relates to the above six areas. One state did not return the questionnaire. (See table on next page.)
<table>
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<tr>
<th>State and Region</th>
<th>Authorities</th>
<th>Lacking</th>
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<td></td>
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<td>Ohio</td>
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<td>Georgia</td>
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<tr>
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<tr>
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### State and Region

#### South Central (Continued)

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<td>Arizona</td>
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<td>2, 4 and 6</td>
</tr>
<tr>
<td>California</td>
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<td>Colorado</td>
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<td>1, 5 and 6</td>
</tr>
<tr>
<td>Hawaii</td>
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<td>Idaho</td>
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<td>Washington</td>
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<td>5 and 6</td>
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<td>Wyoming</td>
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<td>1, 2, 3 and 5</td>
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<td>Puerto Rico</td>
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<td>5 and 6</td>
</tr>
<tr>
<td>Virgin Islands</td>
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</table>

Reported outbreaks of Pullorum disease and Fowl typhoid in 1968 or 1967-68 were received.

#### Chickens

<table>
<thead>
<tr>
<th>Region</th>
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<th>Turkeys</th>
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<tbody>
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<td>3/5 states</td>
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<td>13</td>
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<tr>
<td>5/7 states</td>
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</tr>
</tbody>
</table>
In 1967-68, there were 9,864 chicken flocks containing 33,591,170 birds under the NPIP and 741 reactors or 0.002% reactors were reported. In the same period under the NTIP, 1,386 turkey flocks containing 3,482,049 birds were tested with 19 reactors reported which were not submitted for bacteriological examination. There were 46 states having flocks and hatcheries under the NPIP and 38 states having flocks and hatcheries under the NTIP.

Summary:

There are 18 states that meet all the requirements to enter phase 1 of the proposed PT eradication program for turkeys. Out of the 18 states who qualify for phase 1, probably 16 of the 18 states could enter phase 2 at the end of 1969.

A total of 59 isolations of *S. pullorum* reported from chickens and 6 isolations from turkeys. Three states did not report the number of isolations. There were 15 isolations of *S. gallinarum* reported from chickens and 3 from turkeys. Nineteen states reported isolation of *S. pullorum* and or *S. gallinarum* in 1968 or 1967-68.

<table>
<thead>
<tr>
<th></th>
<th>Chickens</th>
<th></th>
<th>Turkeys</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD  FT</td>
<td></td>
<td>PD  FT</td>
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<tr>
<td><strong>South Atlantic</strong></td>
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<td></td>
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<tr>
<td>3/7 states</td>
<td>3  0</td>
<td></td>
<td>0  0</td>
<td></td>
</tr>
<tr>
<td>1 state no report</td>
<td></td>
<td></td>
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<td>3/6 states</td>
<td>11  9</td>
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<tr>
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<tr>
<td>2/13</td>
<td>14  0</td>
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<td><strong>Puerto Rico and Virgin Islands</strong></td>
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<tr>
<td><strong>TOTAL</strong></td>
<td>59  15</td>
<td></td>
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</table>

19/49 reporting isolations of SP and or SG
3 states not reporting number of isolations
REPORT OF THE COMMITTEE ON
TRANSMISSIBLE DISEASES OF POULTRY

Chairman, H. E. Goldstein, Columbus, Ohio;

The Transmissible Diseases of Poultry Committee met on Tuesday, October 14, 1969 at 7:30 P.M.

A formal paper titled "Pasturella Multocida: Immune Response in Chicks and Mice" was presented by Dr. K. L. Heddleston.

This committee wishes to point out the feasibility of having formal papers presented to the committee printed in our proceedings.

Dr. Ben Pomeroy, chairman of the subcommittee for Pullorum Disease and Fowl Typhoid Eradication, presented his report.

The committee accepted the report as read and recommended that Dr. Pomeroy continue the effort of the subcommittee for the ensuing year. The committee wishes to commend Dr. Pomeroy for his monumental effort in this cause. It has been most frustrating in attempting to finalize the eradication effort. Dr. Pomeroy has led the cause.

The committee as in the past requests that the United States Department of Agriculture provide the mechanics for a national program to eradicate Pullorum disease and Fowl Typhoid.

The committee's attention was directed to the Para Influenza A Viruses as co-related to poultry and man. This committee alerts the industry to possibility of a para-influenza - fowl plague relationship.

The chairman with the concurrence of this committee appointed Ray Bankowski, chairman of the subcommittee to study these relationships and report back to our committee.

Your committee directs the attention of the poultry industry, as well as the chief livestock sanitary officials of each state to the Tentative Guidelines for the licensing of Marek's Disease Vaccine. These guidelines are made available by the Veterinary Biologics Division of the United States Department of Agriculture.

The committee commends the United States Department of Agriculture, Division of Veterinary Biologics for their effort in providing a safe vaccine for Marek's Disease. This committee recommends that an all out effort be made to provide this much needed vaccine as soon as possible.

The incidence of Mycoplasma synoviae (MS) infections has increased this past year. Although the Poultry Inspection Service does not make definitive diagnoses their report shows a continuous problem of 1.5 to 2.9% of all condemnations in broilers. Leukosis is the prime cause of condemnation and has been for some time.
(55% for April, 1969), but synovitis is about fourth in diseases.

The committee directs state regulatory officials attention to ARS 91-74 Bulletin "Mycoplasma Control and Eradication" dated May 1969. This bulletin provides satisfactory guidelines for programming.

This committee suggests that the USDA provide a more critical evaluation of Mycoplasma S6 Antigen; there is an apparent need to re-evaluate stability and expiration dates in relation to the efficacy of the product.

This committee directs the attention of the newly formed committee on Imports - Exports to the needs of controlling the importation of all types of birds and poultry.

The committee commends the action taken in the Duck Virus Enteritis Outbreak in the declaration that the disease was eradicated September 24, 1969. The New York Department of Agriculture, Cornell University, The Long Island Duck Research Laboratory, and the United States Department of Agriculture's three divisions; Animal Health, ADEP, and Veterinary Biologics demonstration that cooperative efforts can provide proper methods for eradication.
SIGNIFICANCE OF CHANGING ECOLOGY ON THE EPIDEMIOLOGY OF ARBOVIRUSES IN THE UNITED STATES

R. P. Hanson and D. O. Trainer
Department of Veterinary Science
University of Wisconsin, Madison

Six arboviruses occurring in the United States are known to induce illness and death in man. Three have been recognized for about 40 years: Eastern encephalitis, Western encephalitis and St. Louis encephalitis. Three have been added or detected in the last decade: California encephalitis, Powassan encephalitis and Venezuela encephalitis. The status of all six are still changing.

Eastern encephalitis was first recognized in Maryland in 1933 and Western encephalitis in California in 1931. Eastern encephalitis was thought to occur only east of the Appalachians and Western encephalitis west of the rockies. Eastern encephalitis is now known to exist west to Texas and Wisconsin and Western encephalitis is found all over North America. St. Louis encephalitis was first recognized in 1932 in Illinois across the Mississippi from St. Louis. It has since been found over most of the country. The extension of the ranges of these viruses does not necessarily mean that the virus has spread, but rather that diagnosticians have looked for them and found them in areas where they had not looked before. One can go back into early journals for accounts of disease recognizable as encephalitis.

An excellent description of what was undoubtedly Eastern encephalitis in horses occurred in Massachusetts and was published in 1823. Similar accounts have appeared every 20 to 30 years. The puzzling disappearances between epizootics make it difficult to clearly establish the geographical range of Eastern, Western and St. Louis encephalitis. Clinical disease in man or horses rarely reappears in any area the following season and may not appear there again for decades. During the intervening quiet years, it may persist in wildlife and its presence can be demonstrated by serologic conversion of certain species of birds; or the virus may disappear entirely based on all of these indicators. The latter has happened during the past four years for Eastern encephalitis in both Wisconsin and Massachusetts.

The three arboviruses, California, Powassan and Venezuela, which were recognized only recently, also have their peculiarities. Powassan virus was first isolated in Canada from the brain of a child that died of encephalitis. This is the only fatal human case known. The virus unlike other arboviruses causing disease in North America, is transmitted by tick bite. It has been isolated from ticks and virus or antibody to it have been found in woodchucks and other rodents in the northern Rockies of our west and in those Canadian border states where it has been sought. No cases of disease in man or horses has been reported in the United States.

California encephalitis is not one virus, but a group of 5 or 6 closely related viruses. The first member of the group was isolated in California from Aedes mosquitoes in 1945. Another member called LaCrosse, was isolated from the brain of a child in 1962. This member of the group occurs in the upper midwest and
regularly causes encephalitis in children. Only a few cases are fatal. Several members of the California group may occur in one geographical area, for example, we find four in Wisconsin. Antibodies to the California group are found in horses, cattle, deer, and a number of wild rodents. It is still not known whether any of the California viruses induce clinical disease in horses.

The last virus to be discussed, Venezuela encephalitis, was first isolated in Venezuela where it incites widespread epidemics in man and horses. While the disease is not commonly fatal in man, it often induces high mortality in horses. In recent years, the disease has appeared in virulent form in Columbia and south into Ecuador and Peru and north into Panama. Milder forms exist north into Mexico. An epizootic foci of the disease exists in Southern Florida where one human case occurred last year. Antibodies have been detected in coastal Texas.

There are many other anthropod borne viruses in the United States, but none of them are known to cause disease in man or livestock. We have 11 arboviruses in Wisconsin and over 120 in the world.

We are principally interested in evaluating what is happening with the six arboviruses we have described. Should we expect serious epidemics of encephalitis in man and horses in the future? Or, will the danger become less?

In all instances, arboviruses are transmitted to man or horses during the warm season of the year by some arthropod; mosquitoes, flies, or ticks. Evidence that biting flies can transmit them is new and was uncovered here in Wisconsin and in Columbia, South America. In one severe epizootic in horses in Columbia, the blackfly, Simulium, was the primary vector of Venezuela encephalitis.

In most instances, species other than man or horses supply the infective blood meal to the arthropod that bites man or horse. This amplifying reservoir is probably a bird in the case of Eastern, Western, St. Louis, and Venezuela encephalitis, and a small mammal for California and Powassan viruses. Still unknown, however, is the animal host that maintains any of these viruses during the period of the year in which arthropods are not active, the overwintering reservoir. Migrating birds and hibernating reptiles and mammals have all been suspected.

Let us examine some environmental factors that affect the abundance of arthropods and the wild species that serve as amplifying hosts. Outside of cities, we are witnessing a change in the landscape. The number of farms and farmers have decreased, and the size of the remaining farms has increased. Farms have become mechanized. The working horse has essentially disappeared. At first glance, one might get the impression that the problem of encephalitis of horses is gone.

Not all of the farm land has been assimilated by the increase in the size of working farms. Near urban areas and in parts of the country where soil is thin or poor or rocky, much land has been abandoned or transferred to recreational use. This is illustrated by an area known to one of us west of Madison were 12 farms in 1919 have become 10 in 1969. However, only four of these are now working farms. The resident human population has fallen from 60 to 30. The horse population has not decreased, but has been transformed from working horses to saddle horses. The last working team disappeared about ten years ago.

The appearance of the land has changed greatly. The open oak parkland covering the steeper hills and constituting about a third of the area of most of the farms was originally used as pasture. As this practice has been abandoned for
chopped hay feeding, the hills have grown up to thick woodland. In some places, abandoned fields are being covered by brush and aspen. The conversion of open land to forest has resulted in a marked increase in such animals as deer and rabbits. It has also provided harborage for arthropods.

The significance of these changes is best illustrated by the prevalence of LaCrosse serotype of California encephalitis. This virus is transmitted to man by the bite of the mosquito *Aedes triseriatis* that breeds only in tree holes. Tree holes are found at the base of oaks that have resprouted after the initial trunk was cut or killed by fire or competition. The cavity formed by the rotting of the old stump collects water in which the larvae develop. Hillsides in the narrow valleys of the upper Mississippi and Ohio Rivers have many trees with these tree holes. The older tree holes were formed by cutting of trees for post and firewood. More recent holes are formed as the smaller trunks are being smothered by competition of neighboring trees. The mosquito remains in the shade of the tree and bites in the early evening. It is a hazard to children in the scenic hills that are being transformed from agricultural to recreational land.

St. Louis encephalitis virus is transmitted by *Aedes pipiens*, a mosquito that breeds in stagnant water of ditches and pools full of organic matter. Sewage polluted drainage ditches near small towns and outflow from improperly constructed septic tanks and manure lagoons provide ideal breeding sites. The problem is increasing, primarily near urban areas.

Not only is the landscape changing and some features of the change increasing the danger for encephalitis, but changes are occurring in the horse and human populations that may also increase the danger of disease. The horse population has been rebuilt to near its original size. Much of it is now concentrated in a belt around urban centers where densities of 100 animals per square mile are not uncommon, but horses are also found throughout all rural areas. The most striking features of the present population is its mobility. Two or three horse shows are held every weekend throughout the summer in most counties of the midwest. Horses are hauled from 5 to 500 miles to all but the smallest shows. Around the larger cities, several shows a season will attract horses from most of the country. In Dane County, horse trailers can be seen from Pennsylvania, Texas, Ontario, and Colorado, as well as neighboring states.

Mobility is also characteristic of the human population. A family from a city can get 2 or 3 hundred miles into the country on a long weekend. Camping in wild areas has increased. The second home in the country, an hour or two drive from the city is not uncommon. The result is a closer association of a large number of people with nature and also with vectors of encephalitis.

The possibility of a man or a horse becoming infected is no longer determined by his legal residence, but by the places where he spends part of his time.

In summary, we find that threat of encephalitis of man and horses difficult to define. The development of better vaccines for some of them and of better diagnosis for all of them seem to be more than offset by the recognition of new members of the encephalitis group, by changes in the environment that favor the vector and by changes in the mobility of man and horses that increase their risk of infection.
REFERENCES

REPORT OF THE PUBLIC HEALTH AND RADIOTHERAPEUTIC COMMITTEE

R. L. Parker, Chairman, Atlanta, Ga.;
R. Fagan, Westchester, Pa.; N. L. Meyer, Hyattsville, Md.; A. B. Park,
Washington, D.C.; I. M. Saturne, Ames, Iowa; Calvin Schwabe, Davis, Calif.;
Robert Singer, College Station, Tex.; J. H. Steele, Atlanta, Ga.; J. H. Stewart,

The committee met at 1:30 p.m., October 14, 1969, and heard two formal
reports and two brief reports which follow. Two recommendations were made
based on the report on toxicology, which will also be submitted to the Committee
on Resolutions. Ten members or guests attended, all expressed a desire to be listed
as members. The following papers were read:

MEAT ANIMALS & PUBLIC HEALTH

A. B. Rich, D.V.M.
Director, Division of Veterinary Public Health
Texas State Department of Health
Austin, Texas 78701

The impact of meat animals on public health, and the susceptibility of man for
certain diseases which are primarily domestic animal problems is a well established
fact, having its scientific foundation laid by Jenner and Pasteur in their work on
cow pox and rabies over a century ago.

In recent years resurgent interest in these matters has occurred giving rise to
increased veterinary participation in epidemiology and research into many diseases
of public health significance. During the last three decades this increased interest
has brought to light many new relationships between the health of animals and
health of man. Many diseases not hitherto known to be intertransmissible between
animals and man have emerged as important factors in public health.

This newer knowledge combined with an increasing governmental concern for
consumer protection has opened an area of responsibility in public health
protection for the livestock and packing industries and the veterinary profession
which is wide in scope and which begs for resolution. The magnitude of the task in
this field seems only partially envisioned by most public health and agriculture
disciplines, and the resolution of the problem is only just begun.

Solutions to the problems have been initiated but they are far from complete.
Certain high points of accomplishment in the field are worthy of mention. During
the past two decades the veterinary profession has been incorporated into public
health as a necessary and constructive discipline for the protection of public health.

More recently and perhaps more directly concerned with protection of public
health in the area of food borne zoonoses, was the passage of the Federal
Wholesome Meat Act in 1967. The demands and the encouragement which this Act
has placed on state governments to move in execution of their responsibilities in
consumer health protection have certainly been heard throughout the land - and the
echoes linger.

The act has engendered movement in the majority of states, which, if nurtured carefully and given time to develop by the federal government, will advance a much needed health protection activity, and perhaps provide a federal-state partnership in government which can serve as prototype for all federal-state relationships. In this respect, the balance is delicate, and present federal legal advantages in this field will require a firm stance in matters of health protection.

And what has this discussion of government and congressional acts to do with meat animal impact on public health? Only nearly everything relating to a complete and successful zoonosis control program. We are experiencing the beginning moves in preventing the diseases of animals from becoming a heavy burden to public health. Through development and growth of a populous and complicated society in the United States, this problem in preventive medicine is becoming increasingly evident. Modern technology in the livestock and meat industries has given us a host of public health problems which we must meet and solve.

For example, the massive feed lot operations which have burgeoned in the last decade do not allow a complacent attitude toward disease control and sanitation in their operation, if they are to be successful and public health is to be protected. Only careful attention to the health of feed lot workers, proper construction of and adequate numbers of employee toilet facilities, and the application of improved sanitation procedures, will prevent repetitive epidemics of cysticercosis in feed lots. The economic loss and risk to public health attendant upon these epidemics is so obvious that elaboration seems unnecessary.

As a further example of the relation of meat animals to public health, let us look at the increase of the salmonellosis problem in recent years. This subject has been so much belabored, that I shall spare you a detailed discussion, but I should like to point to the fact that a major source of human salmonella infections is animals, and that these infections make their way into the human health picture, to a great extent through the many faceted packing industry, and the food, pet, and livestock feed industries allied thereto.

In short, our meat animals are a manifold source of real and potential infections for man. To mention a few more: brucellosis, psittacosis, Q fever, tuberculosis, certain parasitoses and mycoses, and even, perhaps, the possibility that certain malignancies may be infections and transmissible. I could bore you hours on end with specific examples. I hope the two examples I have briefly expanded upon are current enough and big enough to make a point.

As previously indicated, we are on a threshold of accomplishment in zoonotic disease prevention in the area of public health, both human and animal. After nearly a lifetime of work in this endeavor, during which I have seen certain foundations for adequate zoonosis control in construction, I must admit to some hesitancy toward opening the next door for a look at what lies beyond.

However, in more optimistic vein, I would hasten to say that I think the view ahead will be brighter than that behind us. With understanding and careful cooperative regulation by the various government jurisdictions which are involved at all levels of government, and by a similar understanding and cooperative effort by the traditionally progressive American livestock and food industries, I think the problems will be solved.
GENERAL COMMENTS

The Iowa State University Veterinary Diagnostic Laboratory serves the state of Iowa in the diagnosis of disease problems in animals including those caused by pesticides, environmental pollutants, feed additives, rodenticides, and toxic plants. Our Toxicology Section was begun approximately 4½ years ago by the forming of an analytical-chemistry-toxicology group made up of chemists and veterinarians spending full time on the identification and interpretation of problems encountered with pesticides, economic poisons, and other toxicants. We presently have a staff of five veterinarians and five chemists working on toxicology problems.

We have come to the conclusion that the use of organic insecticides for agricultural purposes is not hazardous when they are handled as recommended.

It is mandatory that those in the business of producing food and fiber have a means of controlling the pests that attack their crop or product. At the present time, insecticides and other pesticides are the only practical means of controlling damage to food and fiber by pests. Banning the use of any pesticide should be considered from all aspects before such action is taken. There must be consideration of the beneficial effects as well as the known harmful effects resulting from insecticide use. We must not be guilty of banning the use of a chemical because of public opinion, mass hysteria, or emotion. However, in those areas in which a hazard is obvious and where there is opportunity for preventing such hazard to man, plants, and animals, we should take action. With this in mind, we are making two recommendations.

In recent years, drugs, pesticides, nutrient substitutes, and other chemicals have been added to animal feeds in an effort to more efficiently and economically dispense these agents to livestock and poultry. Also, many feed companies distribute fertilizers and agricultural chemicals not intended for mixing in feeds. With the advent of these practices have come problems, either due to human error or otherwise, which have resulted in losses for which the manufacturer is liable; and beyond that, there has been chemical contamination of the public meat supply in the form of residues resulting from excessive exposure of animals to pesticides and drugs.

Two problems which we frequently encounter at the Iowa Veterinary Diagnostic Laboratory include (1) contamination of animal feeds with granular and powdered formulations of insecticides and (2) the use of arsenic as a herbicide. In
both of these problem areas there has been great economic loss to livestock producers; but more importantly, there is a potential public health hazard in each case.

**PESTICIDES IN ANIMAL FEEDS**

Accidental incorporation of massive levels of insecticides in animal feeds is a primary source of the pesticide problems in livestock. This may occur as a result of mis-identification by the farmer, who has previously stored granular or powdered insecticides in the feed area and subsequently added it to a grain mixture, thinking it to be minerals; or it may occur as a result of error by the local elevator and even possibly by the feed manufacturer. In Iowa alone during the past three years, we have investigated and documented on the average of two such episodes per month involving corn and soybean insecticides including aldrin, heptachlor, diazinon, thimet, and other organochlorine and organophosphate insecticides compounds. The massive contamination of animal feeds naturally results in acute death of some of the animals being fed the mixture; but, more importantly, extremely high levels of insecticide may be accumulated in the fatty tissues and remain there at the time of slaughter for human consumption. Our laboratory has documented cases in which carcasses contained between 40 and 50 ppm insecticide in the fat of beef slaughtered for human consumption.

Oft-times a large number of animals may be killed as a result of a massive exposure. These animals may contain 2-3,000 ppm insecticide in their rumen contents. The rumen of a 1,000-pound steer may contain as much as 200-250 pounds of feed. One can see that the use of several animals that have died of massive pesticide exposure for tankage, which subsequently will be fed to poultry, swine, and perhaps dairy animals, is a potential source of insecticides in eggs, pork, and milk. We have also investigated and documented such cases in swine in which massive doses of insecticide have been mistakenly added in place of a mineral mixture, resulting in a heavy death loss plus contamination of the meat slaughtered for food.

A specific example is as follows: An Iowa farmer had 95 head of 550-pound steers in a feedlot. He purchased feed from the local elevator which included corn cobs, urea, and cracked corn. The next morning following delivery of feed on the previous evening, the farmer went out to find 35 head down in the mud dying in convulsive and central nervous system involvement. Thirty-six died and the remainder recovered and were fed out for slaughter. Chemical investigation revealed 2,000 ppm aldrin in the stomach contents of the dead animals. The fatty tissues contained up to 58 ppm dieldrin in those animals that died. In tracing back the sequence of events which led to the massive contamination of the feed, it was found that the local elevator stored a large pile of corn cobs in a warehouse in which a partition holding the cobs in place consisted of a stack of 50-pound bags of 20 percent aldrin granules. In scooping up the corn cobs with a tractor, the operator had inadvertently broken the bags of aldrin releasing the granules into the cobs. Needless to say, the local elevator was responsible for the damages. Not only was there a severe death loss and a setback to the affected animals, but perhaps even more importantly we know that 36 carcasses containing high levels of aldrin
were processed into tankage for animal feeds. In addition, we know that the animals that recovered and were subsequently slaughtered for human consumption after several months, contained significant levels of dieldrin, the epoxide of aldrin.

RECOMMENDATION

Such episodes as the one just related commonly occur in our state, and there is evidence that they occur throughout the cattle and swine feeding states. These accidents always get widespread publicity by news media. They create a distrust by the consuming public and give the use of agricultural chemicals and drugs a black eye. It is our contention that if these episodes could be reduced, we would go a long way toward eliminating the fear that the public has of using pesticides and other chemicals which are so necessary for the production of food and fiber. We, therefore, are recommending that hazardous insecticides prepared in granular or powdered form for agricultural purposes be identified by the incorporation of a dye marker such as charcoal, graphite, or some other readily identifiable material that would immediately show up if it were incorporated in animal feeds.

The Agricultural Chemical Committee at Iowa State University has recommended to the Iowa State Legislature that the Department of Health, Education, and Welfare (and I might add, the U.S. Departments of Agriculture and Interior) be requested to hold a hearing on the feasibility of color-identifying granular and powdered insecticides prepared for agricultural purposes. Such a practice would help eliminate the opportunity for massive exposure of livestock and certainly would help the feed manufacturer prevent accidental contamination of his product.

ARSENICAL HERBICIDES

During the past five years, the Iowa Veterinary Diagnostic Laboratory has encountered numerous problems associated with the common practice of using commercial preparations of arsenical herbicides. There are many preparations presently being sold ranging from crab grass control for lawns in towns and cities, to control of weeds along county roads, school yards, highway right-of-ways, and for removal of thistles and other noxious shrubbery. Commercial preparations containing arsenic recommended as herbicides contain up to 50 percent arsenic trioxide or sodium arsenite. Others contain arsenate of lead. These preparations are very toxic to pet animals, livestock, birds, and humans.

The following examples of poisoning in animals and potential poisoning in humans have occurred:

1. (1969) The backyards of several homes in West Des Moines adjoin to form a small 1-2 acre pasture in which several of the families maintained pleasure horses. One of the backyards was treated with a crab grass control containing 47 percent arsenic trioxide and 3½ percent arsenate of lead. Approximately three weeks later, after several rains and after the lawn had been watered, the grass was clipped with a lawn mower and the clippings thrown over the fence to the horses. The horses readily ate the clippings and within a period of four days a total of five horses were killed. The grass clippings contained 6 percent metallic arsenic.
2. (1969) A janitor of a local high school used a commercial preparation of sodium arsenite as instructed, spraying the periphery of a football field including a fence row adjoining a cattle pasture. A few hours later the cattle ate the grass contaminated with arsenic, eating a strip of approximately 8 inches wide next to the fence. A total of five cows were killed and four were affected but recovered.

3. (1965) A caretaker for the cemetery sprayed the fence row with a sodium arsenite commercial preparation allowing the spray to drift for a few inches over into an adjoining cattle pasture. A total of 15 dairy cows were killed. The dairyman and the farm family consumed milk from an affected cow and arsenic poisoning was diagnosed in one member of the family.

4. (1968) A commercial crab grass control formulation containing arsenic trioxide was applied in the fall of the year to grass in a backyard. The next spring, at least four months later, the grass was clipped and the clippings thrown over the fence to calves which subsequently ate the clippings resulting in the death of several animals.

In every above instance, the level of arsenic in the grass after the application of the arsenical herbicides was extremely high, up to 6 percent (60,000 ppm metallic arsenic). It has become obvious that the arsenical herbicides are extremely toxic to animals. In one case a dog was poisoned after eating some of the blades of grass following the application of an arsenical crab grass control preparation. Livestock apparently like the taste of arsenic and will seek out soil and plants sprayed with an arsenical preparation. Invariably this results in death to many animals. Humans are susceptible to arsenic, and it is quite feasible that a small child could drop a candy sucker into the grass, pick it up, and enough arsenic be present to poison the child. Such occurrences would not be immediately obvious to a physician and severe illness and death could occur without the physician being aware of the cause. Such cases can only be diagnosed with the aid of analytical chemical procedures such as are available at the Veterinary Diagnostic Laboratory at Iowa State University.

RECOMMENDATION

It is our recommendation that arsenicals be banned for sale and use as a herbicide. There are many good organic herbicides that can be used. Apparently, the only reason for the use of arsenical herbicides if that they are less costly than most presently recommended organic herbicides. Frequently local governments such as school boards, county supervisors, and perhaps the state highway commission utilize the arsenical herbicides. Also, certain commercial firms use arsenicals to kill weeds in areas such as electrical highline right-of-ways and substation plots. The general population is not aware that arsenical herbicides are extremely toxic to humans and animals whereas most other organic herbicides are relatively nontoxic.

A lively and lengthy discussion followed Dr. Buck's presentation. The Committee on Public Health and Radiological Fallout recommends that the United States Animal Health Association request that the Consumer Protection and Environmental Health Administration of the United States Department of Health,
Education and Welfare, the Agricultural Research Service of the United States Department of Agriculture, and the Division of Research of the United States Department of Interior hold hearings on the adoption of methods of color identification of granular and/or powdered insecticides which might be used for agricultural purposes, as a means of preventing accidental contamination of animals to be used as human food with potentially deleterious chemicals. The committee on Public Health and Radiological Fallout recommends that the United States Animal Health Association transmit a copy of the portion of the committee's report on Pesticides and Economic Poisons in the Food Chain and the preceding recommendation to the Secretary, United States Department of Health, Education and Welfare as a statement of the Association's support of his committee on the development of a national policy on pesticides in relation to food, the environment and the public health.

Dr. Parker summarized the number of cases of human listeriosis reported to the National Communicable Disease Center for 1968. Cases reported increased from 60 in 1967 to 105 in 1968. At least 24 of the 1968 cases were fatal. The greatest number of cases was in infants less than one year of age in whom a case fatality rate of 24% was observed. In patients over 40 years of age the case fatality rate was 77%. Only four cases, with no fatalities, occurred in the 1 to 40 year old age groups. Onset, judged by date recorded or date of first culture, occurred during the summer and early fall months in 60% of the cases.

Attention was called to the professional teamwork necessary in defining sources of infection of many of the zoonoses. Examples of the current cooperative attitude are the policies in effect for tracing meat borne outbreaks of food poisoning beyond the packing plant of origin and the program for tracing brucellosis to herds of origin when cases in man are reported.

The meeting adjourned at 4:30 p.m. The Committee respectfully submits this report for consideration and acceptance.
PROGRESS IN RABIES RESEARCH

R. K. Sikes, D.V.M., M.P.H.

Significant rabies research has taken place during recent years in several laboratories in the United States and other countries of the world. In this paper, I will summarize three specific areas of the research which have the most direct application in rabies control and diagnosis; human treatment, animal vaccine evaluation, and fluorescent antibody application in the corneal test.

HUMAN TREATMENT

The two types of biologics which are now used in persons exposed to the bites of rabid animals are effective but both cause undesirable reactions in thousands of people in the United States each year. Therefore, research at the National Communicable Disease Center and at the Wistar Institute, both WHO Rabies Reference Centers, has been toward developing prototypes of potent and non-allergenic biologics to be used ultimately for post-exposure prophylaxis.

Rabies Immune Globulin (RIG), Human Origin — Several experimental lots of RIG have been prepared from the plasma of hundreds of veterinarians and other volunteers who had rabies antibody. The following accomplishments have been realized on this program: 1, 2, 3.

1. Pure gamma IgG globulin of human origin has been prepared with resulting rabies antibody titers higher than that required of the present equine origin antirabies serum. The last lot prepared at NCDC contained 166 international units per ml; minimum standards now require 100 units per ml.
2. All lots were fractionated by the Cohn ethanol procedures and they passed all the requirements for use in man.
3. The RIG experimental animals that had been challenged prior to treatment as well as horse origin antirabies serum.
4. Homologous origin antirabies serum required less antibody than heterologous serum to protect animals challenged with rabies.
5. RIG has recently been administered to 24 human volunteers to obtain an antibody profile or half-life of this homologous rabies antibody. The antibody persisted over 56 days in all who received the recommended dose. Antibody was still detectable after 70 days in several volunteers, or 4 to 5 times longer than in persons who received equine origin antirabies serum (ARS). No undesirable reactions resulted from the use of RIG.
6. Another human study is planned for the Fall of 1969 and should be completed by early 1970. This was designed to determine the degree of interference that might result and the regimens which might be used with this homologous globulin.
7. This type of product (RIG) now seems feasible to prepare and it is possible that one or more commercial laboratories will prepare it for use in the United States within the next 2 years.

Human Vaccine Research — In the United States and Canada, there has been considerable progress in developing more potent and safer vaccines for use in
humans. Except for the duck embryo vaccine which has been widely used in this country since 1958, there have been no significant changes in rabies vaccines used in the United States since the 1880's. Now, techniques for preparing more purified and concentrated rabies vaccines have been developed and the results published. It is now possible to prepare vaccines that are purified and concentrated. They are much more antigenic than the types currently being used and are freer from measurable biologic impurities. Several of these newer vaccines are being tested in primates in our Lawrenceville facilities at NCDC now. Antigenic values of some are over 50X, the current requirements, and the antibody titers are much higher in animals given an injection of certain of these newer vaccines than when the older types are used.

It is generally conceded by rabies researchers that the future rabies vaccines of tissue culture origin which are purified and concentrated will be more potent and safer, thus making it feasible to consider reducing the number of doses of vaccine used in the post-exposure treatment series.

ANIMAL VACCINE EVALUATION

Excellent animal rabies vaccines have been available in the United States since the early 1950's, as has been well established as based on laboratory development and tests which reflect the immunogenicity of the vaccines. Further proof of the merits of these vaccines has been the fact that dog rabies in the United States has been reduced from over 8,000 cases per year in the 1940's to only 296 cases in 1968.

During recent years several types of tissue culture vaccines have been developed in different laboratories and have been licensed (Table I). All of the vaccines listed pass the required potency and safety tests of the Veterinary Biologics Division, United States Department of Agriculture, at the National Animal Disease Laboratory (NADL).

Since there was few data available on long duration of immunity studies regarding most of these newer vaccines, the state and territorial public health veterinarians and an NCDC rabies advisory panel indicated the need to have one major study to determine the relative immunogenicity of these newer tissue culture vaccines. Thus, five tissue culture and one low egg passage chick embryo types were selected by the NADL and NCDC staffs to be compared for their ability to immunize purebred beagles in a three-year duration-of-immunity study. In addition, a purified rabies vaccine from NCDC and a suckling mouse brain vaccine which is used in Latin America and provided by the Pan American Health Organization are being compared.

Results are completed on antibody titers for the first two years and on the challenge for the first year. The third year challenge will take place in May, 1970. There is a strong suggestion from results obtained thus far that inactivated vaccines with less antigenic mass are not as immunogenic as the modified live tissue culture or CEO types.

This is especially apparent when the vaccines are compared on the basis of antibody. Peak antibody titers were obtained in each of the 40 animals per vaccinated group between 2 and 4 weeks after vaccination. The median titers
ranged between 1:70 and 1:625 per vaccinated group at that time. After one year the median titer of only one vaccinated group had no detectable antibody, namely those dogs vaccinated with the inactivated tissue culture type. The same was true for the dogs after two years when 7-S antibody is persistent. The only group whose median titer was less than 1:2 was from the inactivated, tissue culture vaccinated group. No challenge was conducted after 2 years from vaccination and only 10 per group were challenged one year after a single injection of vaccine was administered. In that challenge comparing the commercial vaccines, 10 of 10 unvaccinated controls died of rabies and only 4 of 60 vaccinated dogs died of rabies. Three of these 4 were from the group of 10 vaccinated with the inactivated, tissue culture type of vaccine. One of 10 vaccinated with the modified live LEP-hamster kidney tissue culture type died, but none of the remaining dogs vaccinated with any of the 4 live vaccines died.

At this time, it must be stated that on the basis of one small challenge group, there was no statistically significant difference observed among any of the vaccinated groups of dogs. Final statements on these vaccines will be made in 1970 when this study is completed.

**FLUORESCENT ANTIBODY (FA) APPLICATION, CORNEAL TEST**

Dr. L. G. Schneider, from Tubingen, Germany, recently reported on an intra-vitam rabies test - the application of FA using corneal impressions to diagnose rabies in the live, rabid animals\(^9\). His first publication presented results in mice inoculated intracerebrally or by the volar route.

In his first experiments, Schneider worked mainly with experimentally-infected mice although he did examine several foxes also. He reported obtaining a rabies specific diagnosis on more than 70% of infected mice prior to the onset of clinical symptoms. He also found that none of the infected mice that were negative by the corneal FA test contained virus in their salivary glands. This test would therefore seem to offer a promising method to prove the non-infectivity of salivary glands.

At NCDC, Dr. R. G. Arko has confirmed much of Schneider's work in mice and has also had some corneal FA positives in a few other animals. Further evaluation of this technique is being pursued at NCDC.
TABLE 1
TISSUE CULTURE RABIES VACCINES AVAILABLE IN THE UNITED STATES

<table>
<thead>
<tr>
<th>CELL TYPE</th>
<th>VIRUS STRAIN</th>
<th>SPECIES IN WHICH VACCINE IS LICENSED FOR FIELD USE</th>
<th>RECOMMENDED DOSE</th>
<th>VACC. ROUTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Modified Live</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine Kidney</td>
<td>ERA</td>
<td>Dogs, Cats, Cattle, Sheep, Goats, Horses</td>
<td>2 ml., all species</td>
<td>Intramuscular (I.M.)</td>
</tr>
<tr>
<td>Canine Kidney</td>
<td>HEP-F1.</td>
<td>Dogs, Cats, Cattle</td>
<td>1 ml.</td>
<td>I.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 ml. - 1 ml.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>in 2 doses</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 wks. apart</td>
<td></td>
</tr>
<tr>
<td>Hamster Kidney</td>
<td>LEP-F1.</td>
<td>Dogs only</td>
<td>2 ml.</td>
<td>I.M.</td>
</tr>
<tr>
<td>Chick Embryo</td>
<td>LEP-F1.</td>
<td>Dogs</td>
<td>1 ml.</td>
<td>I.M.</td>
</tr>
<tr>
<td>B. Inactivated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster Kidney</td>
<td>CVS</td>
<td>Dogs</td>
<td>5 ml.</td>
<td>Subcutaneous (S.C.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cats</td>
<td>3 ml.</td>
<td>or I.M.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cats</td>
<td>50 ml.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sheep, Goats</td>
<td>10-15 ml.</td>
<td></td>
</tr>
<tr>
<td>HKTC with Adjuv.</td>
<td>CVS</td>
<td>Dogs</td>
<td>2 ml.</td>
<td>S.C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cats</td>
<td>1 ml.</td>
<td>or I.M.</td>
</tr>
</tbody>
</table>
REFERENCES


RABIES IMMUNITY IN VACCINATED CATTLE*

Presented at the 73rd Annual Meeting
U.S. Animal Health Association
October 13, 1969
by
Harold B. Hubbard, DVM, MPH
Veterinary Medical Services
Pan American Health Organization
Washington, D.C.

Authors: Eduardo Fuenzalida, DVMa; Pedro N. Acha, DVM, MPHb; Pascu Atanasiu, MDC; Oscar Larghi, D.Sc.a; Boris Szyfres, DVMa.

SUMMARY
A series of rabies vaccines were studied for the purpose of determining their capacity to induce the formation of rabies neutralizing antibodies, as well as their ability to protect the vaccinated animals against a challenge with a rabies strain originated from vampire bat.

INTRODUCTION
Rabies in cattle transmitted through bites of infected vampire bats has reached proportions of such magnitude in some countries of Middle and South America that it constitutes a limiting factor for livestock production. It is estimated that more than half-a-million cattle die of rabies every year in these countries, with an economic loss of over $70,000,000.3,16

Reduction and control of the transmitting vector, the bat, has not been an effective measure8 and until control methods are much improved immunization of exposed animals appears to be the only means of solving the problem.

Immunization of cattle with vaccines prepared by various methods has not always produced satisfactory results. Which vaccine or vaccines are best for use in cattle still remains to be determined.7,17,18

Live attenuated virus vaccine, propagated either in chick embryo or in cell culture systems, is one type that has been used2,14. The immunization mechanism with live vaccines is based on multiplication of the rabies virus in tissues of the vaccinated animal, with immunity developing as a response to a modified infection. This concept has been generally accepted, however, additional investigation is needed to clarify the action of all rabies virus vaccines. The other type of vaccines are those produced with inactivated rabies virus. In vaccines of this type the production of immunity is based on the quality and quantity of the antigen they contain. In this case there is no multiplication of virus and the vaccinated animal receives a single antigenic stimulus upon which the establishment of immunity

(a) Pan American Zoonoses Center, PAHO/WHO, Buenos Aires, Argentina.
(b) Veterinary Medical Services, PAHO/WHO, Washington, D.C., USA
(c) Pasteur Institute, Paris
depends.

It is known that in rabies there is a direct relation between resistance to experimental infection and presence of antibodies in the blood\textsuperscript{6,9,20}. This has been observed in vaccinated mice, guinea pigs, dogs and cattle which have been exposed to experimental infection. However, vaccinated dogs and cattle can resist experimental infection without detectable antibodies in the serum.

The present study comparing live and inactivated vaccines was conducted as part of a more extensive investigation of rabies vaccines. The objectives were to determine the ability of each vaccine to stimulate the formation of neutralizing rabies antibodies and to provide protection to the vaccinated animals against challenge with a highly infective rabies virus strain of vampire bat origin.

MATERIALS AND METHODS

\textbf{Cattle.} The cattle used were Aberdeen Angus, between the ages of one and two years. All of the animals were raised on the Experimental Farm of the Pan American Zoonoses Center, located in an area reported to be free of rabies.

\textbf{Guinea pigs.} The guinea pigs used in the experiment were American short hair.

\textbf{Mice.} The white laboratory mice used in the experiment were strain CF-1.

\textbf{Vaccines.} Three of attenuated live virus vaccines and three of inactivated fixed virus, were tested in 8 groups of cattle (See Table I). Potency of each vaccine was determined in our laboratory using procedures described by the World Health Organization and the U.S. Department of Agriculture, VBD. All passed the minimum potency requirements.

\textbf{Virus used to challenge cattle.} DR 19 obtained from the Instituto Biologico, Rio de Janeiro, Brasil. This strain was originally isolated from vampire bat \textit{(Desmodus rotundus rotundus)} and has been passaged 19 times I.C. in mice. At this stage this virus has a titer of about 10\textsuperscript{6.55} LD\textsubscript{50}/0.03 ml. in mice.

\textit{Experiment 1.} ERA vaccine propagated in pig kidney culture (ETA)\textsuperscript{1} was used in this experiment. Eight cows received a first dose of 2 ml. of vaccine intramuscularly in the heavy muscles of the rear limbs. A total of 1 ml. was injected intradermally in the cervical region as a booster thirty days after. (Group A)

Eight other cows (Group B), received a single dose of 2 ml. of the same vaccine intramuscularly in the heavy muscles of the rear limbs.

\textit{Experiment 2.} One lot of the high egg passage, Flury strain of chick embryo origin (HEP-CEO)\textsuperscript{13,14,15} was tested in twenty (20) cattle in this experiment (Group C) using an intramuscular injection of 5 ml.

Eight other cows (Group D), received a single intramuscular dose of 5 ml. of the same lot of vaccine reconstituted with a diluent that contained 9.1% of Al (OH)\textsubscript{3} (HEP-CEO + Al).

\textit{Experiment 3.} Flury strain live virus (HEP-CEO) further attenuated by propagation in dog kidney epithelial cells (HEP-DKC)\textsuperscript{5,21} was tested by IM injection of 2 ml. into each of 10 cows (Group E).

\textit{Experiment 4.} Vaccine used in this experiment was a 5% suspension of suckling mouse brain (SMBV)\textsuperscript{12}. The virus was inactivated by UV light.

Ten cows (Group F), received a first dose of 10 ml. and a booster of 5 ml. thirty days afterwards, by the intradermal route, distributed in various sites in the cervical region.
Experiment 5. A fixed virus vaccine prepared with 10% brain suspension of new born lambs (SLBV) and inactivated with betapropiolactone was used to inoculate eight heifers (Group G), from three to eight months of age with a single dose of 5 ml. subcutaneously.

Experiment 6. In this experiment a fixed virus vaccine was prepared at 4% concentration from brain tissue of suckling mice and inactivated by ultra violet light.

The product was lyophilized, and at the time of use, it was reconstituted in an Al(OH)₃ gel which contained 10 mg. of Aluminum Oxide per ml. (SMBV + Al)₁₀. Ten cows (Group H), from eight months to one year of age, received a single dose of 10 ml. of the absorbed Al(OH)₃ vaccine subcutaneously in the cervical region.

Serology — Samples were obtained from cows of Groups A, B, C, D, and F, on the day of the initial vaccination and at 30, 38, 100, 200 and 365 days following the first vaccination. The same periodicity was maintained in the other groups, except for the sampling on the 38th day which was omitted.

Each animal was bled from the jugular vein; 20 to 50 ml. of blood was obtained each instance. The sera was removed, inactivated for 30 minutes at 56°C. and stored at -20°C.

Serum neutralization tests were carried out according to the technique described by Atanasiu, et al.³. Three or five fold dilutions were made with the inactivated sera. The final serum dilutions ranged between 1:2 and 1:486 when three fold dilutions were made, and between 1:5 and 1:625 when five fold dilutions were made. The titer of the CVS virus used in the test ranged from 20 to 75 LD₅₀.

Challenge — The vaccinated cattle were divided into two challenge groups. In the first challenge four or five animals of each of the vaccine groups A and B, C and D, E, G and H, were injected with 2 ml. in the masseter muscle of a 10⁻² dilution of virus (containing 2 x 10⁶ ICMLD₅₀). In the second challenge study, four or five cattle of the groups A and B, C and H, were injected with 2 ml. of a 10⁻¹.₃ dilution of virus (containing 10⁷ ICMLD₅₀). This selection was based on the homogenous distribution according to the previous S-N titers.

In the first challenge the interval after vaccination ranged from 300 to 930 days; in the second challenge, the interval after vaccination ranged from 480 to 1,050 days.

RESULTS

Individual SN antibody titer obtained with the various antirabies vaccines are shown in Tables II through IX. The animals exhibited no unfavorable reactions or signs of rabies as a consequence of the vaccination.

None of the sera of the 82 heifers receiving the vaccines had rabies neutralizing antibodies on the day of inoculation of the first dose of the vaccine.

Neutralizing antibodies were detected on day 30 in all the animals except those in the group that received the HEP-DKC vaccine (Group E — Table VI).

Application of a booster dose 30 days following the initial vaccination stimulated the neutralizing antibody titer response within a period of eight days (Group A and F) the shortest period tested. However, this booster dose did not
influence the persistence of antibodies since they declined rapidly to their initial level (compare groups A and B). This was observed in the animals vaccinated with ERA vaccine (Group A) and with the SMBV vaccine (Group F).

Peak antibody titers were elicited after 30-38 days; but declined rapidly in several groups. This was most obvious in animals vaccinated with HEP-CEO and HEP-CEO + Al vaccines (Groups C and D). Antibody persisted for two years in all the animals given either a single or double dose of ERA vaccine. The same difference is also evident for the various groups comparing the profile of the median antibody titers (Table X).

A summary of the results of the first challenge experiment is presented in table XI. All vaccinated animals of groups A, B, D, and H, withstood the challenge. One of four in Group G, and three of five on Group E died of rabies, whereas four of the five unvaccinated controls died of rabies.

Table XII shows the results of the second challenge experiment. In this test, all vaccinated animals of groups A and B survived the challenge at 1,050 days. None of those vaccinated with HEP-CEO survived the challenge at 810 days. All of those vaccinated with SMBV + Al (OH)₃ survived when challenged at 480 days.

DISCUSSION

Detectable antibodies were present in serum of all animals vaccinated 30 days before with the exception of group E (HEP-DKTC). Serums of Groups A (ERA), B (ERA) and H (SMBV + Al) had high antibodies titers up to a year. In Group D (HEP-CEO + Al), however only two animals had SN antibodies, and at a very low level.

Appearance and persistence of antibodies at a constant level during an extended period, shown in Groups A and B indicate that immunity persisted during this same period. This was not observed in Group E (HEP-DKTC) and in Group F (SMBV), where the two vaccines used contained a reduced amount of antigen. Antibody decreased rather rapidly even with the application of a booster dose as demonstrated in Group F.

Animals from Groups A and B revealed antibodies in the constant form after a decline in the first few days. In some of them as revealed in Tables II and III, a rise in the antibody titer without additional antigenic stimulus was observed 1,050 days after date of the original vaccination. This might indicate that the inoculated live attenuated virus from the vaccine continued to live in the organism and was capable of giving a constant stimulus. It is possible that the inoculated virus remained in a symbiotic form in some of the cells, thereby being protected from the neutralizing action of the circulating antibodies. In this way, each time that the circulating antibodies were diminished to the point of extinction, the infected cells became capable of supplying new charges of virus with resultant production of new antibody. Or more specifically, the viral nucleic acid may be free from the protein and infect cells. The transmission of infective nucleic acid may be rapid between adjacent cells or under the influence of a protective device to prevent enzyme inactivation. Another postulation could be that a mechanism exists for the production of enzyme inhibitors during the transmission between cells.

The mechanism appears to be completely inhibited in the case of Group D, where the modified live virus is absorbed with Al (OH)₃ and for this reason
probably failed to enter any cells (Table V). The first immune response corresponds to a reaction of the mobile elements of the R.E. system. In such a way, the addition of adjuvants appears to upset the immunogenic mechanism in the live virus vaccines or response of the host especially if we consider that the same kind of vaccine without Al (OH)_3 gave a prolonged immunogenic reaction and protected the cattle 660 days post-vaccination (Group C).

With the inactivated vaccines, production of antibodies is general but subject to decline. This decline probably indicates a depletion of introduced antigen after a short time (Group F and G). Decline of titer is slower when adjuvant is combined with the inactivated vaccine as in the case of Group H19.

Post-challenge survival corresponded well with the presence of antibodies. This is shown by the results of the challenge of these experiments in which the survival rate corresponds directly to the presence of detectable antibodies, even at a minimal level (1:2 final dilution of serum).

It appears that appreciable resistance to challenge persists even when serum antibody levels have decreased to non-detectable levels in animals with previously detectable antibodies. Probably the challenge inoculation acts as a booster with quick antibody response although this was not established in these experiments.
### TABLE I
Vaccines used

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Vaccine used</th>
<th>Method of propagation</th>
<th>Inactivated by</th>
<th>Group</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ERA with booster</td>
<td>Pig kidney tissue culture</td>
<td>-</td>
<td>A</td>
<td>Vaccinated 8</td>
</tr>
<tr>
<td></td>
<td>ERA</td>
<td>-</td>
<td>-</td>
<td>B</td>
<td>Challenged 5</td>
</tr>
<tr>
<td>2</td>
<td>HRP-CBO</td>
<td>Chick embryo</td>
<td>-</td>
<td>C</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>HRP-CBO + Al (OH)₃</td>
<td>-</td>
<td>-</td>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>HRP-DKTC</td>
<td>Dog kidney tissue culture</td>
<td>-</td>
<td>E</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>SMBV with booster</td>
<td>Suckling mouse brain</td>
<td>U-V irradiation</td>
<td>F</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>SLBV</td>
<td>Suckling lamb brain</td>
<td>Beta-propiolac-tone 1:4000</td>
<td>G</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>SMBV + Al (OH)₃</td>
<td>Suckling mouse brain</td>
<td>U-V irradiation</td>
<td>H</td>
<td>10</td>
</tr>
</tbody>
</table>

### TABLE II
Group “A"
Serum Neutralizing Antibody Titers in Cattle Vaccinated with two doses of the ERA Porcine Kidney Tissue Culture Vaccine

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>132</td>
<td>0</td>
</tr>
<tr>
<td>135</td>
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<tr>
<td>142</td>
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<tr>
<td>145</td>
<td>0</td>
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<td>149</td>
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</tr>
<tr>
<td>159</td>
<td>0</td>
</tr>
<tr>
<td>160</td>
<td>0</td>
</tr>
</tbody>
</table>

* Bleeding eight days after the booster dose.

a- Titers on day of virus challenge as indicated in Table XI.

B- Titers on day of virus challenge as indicated in Table XII.
RABIES IMMUNITY IN VACCINATED CATTLE

**TABLE III**

*Group “B”*
Serum Neutralizing Antibody Titers in Cattle Vaccinated with one dose of the ERA Porcine Kidney Tissue Culture Vaccine

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Days</th>
<th>0</th>
<th>30</th>
<th>38</th>
<th>100</th>
<th>200</th>
<th>365</th>
<th>720</th>
<th>930</th>
<th>1050</th>
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</thead>
<tbody>
<tr>
<td>161</td>
<td>0 &gt; 25</td>
<td>125</td>
<td>46</td>
<td>40</td>
<td>42</td>
<td>30</td>
<td>70b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>0 &gt; 25</td>
<td>56</td>
<td>68</td>
<td>32</td>
<td>12</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>0 &gt; 25</td>
<td>56</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>0 &gt; 25</td>
<td>32</td>
<td>32</td>
<td>19</td>
<td>10</td>
<td>54</td>
<td>162b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>171</td>
<td>0 &gt; 25</td>
<td>37</td>
<td>40</td>
<td>56</td>
<td>10</td>
<td>8</td>
<td>8b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>172</td>
<td>0 &gt; 25</td>
<td>56</td>
<td>40</td>
<td>19</td>
<td>37</td>
<td>40</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>173</td>
<td>0 &gt; 25</td>
<td>68</td>
<td>56</td>
<td>56</td>
<td>42</td>
<td>54</td>
<td>31a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>174</td>
<td>0 &gt; 25</td>
<td>19</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a- Titers on day of virus challenge as indicated in Table XI.
b- Titers on day of virus challenge as indicated in Table XII.
TABLE IV

Group "C"

Serum Neutralizing Antibody Titers in Cattle Vaccinated with one dose of attenuated live virus vaccine of chick embryo origin (Flury HEP strain).

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>175</td>
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<td>191</td>
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<td>192</td>
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<td>195</td>
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</tr>
<tr>
<td>196</td>
<td>0</td>
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<tr>
<td>202</td>
<td>0</td>
</tr>
<tr>
<td>203</td>
<td>0</td>
</tr>
</tbody>
</table>

a- Titers on day of virus challenge as indicated in Table XI.
b- Titers on day of virus challenge as indicated in Table XII.
TABLE V

Serum Neutralizing Antibody Titers in Cattle Vaccinated with one dose of attenuated live virus vaccine of chick embryo origin (Flury HEP strain) with Al(OH)₃

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>181</td>
<td>0</td>
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<td>183</td>
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<td>215</td>
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</tr>
<tr>
<td>218</td>
<td>0</td>
</tr>
</tbody>
</table>

a- Titer on day of virus challenge as indicated in Table XI.
TABLE VI
Serum Neutralizing Antibody Titers in Cattle Vaccinated with one dose of live virus vaccine prepared in dog kidney cells (Flury HEP strain)

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>225</td>
<td>0</td>
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<td>236</td>
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<td>258</td>
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<tr>
<td>260</td>
<td>0</td>
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</tbody>
</table>

a- Titers on day of virus challenge as indicated in Table XI.
TABLE VII

Group "F"
Serum Neutralizing Antibody Titers in Cattle Vaccinated with two doses of inactivated fixed virus vaccine prepared from suckling mouse brain.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>139</td>
<td>0</td>
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<tr>
<td>146</td>
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<td>166</td>
<td>0</td>
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<tr>
<td>167</td>
<td>0</td>
</tr>
</tbody>
</table>

* Bleeding eight days after the booster dose.
TABLE VIII
Group "G"
Serum Neutralizing Antibody Titers in Cattle Vaccinated with
one dose of fixed virus vaccine prepared from newborn
lamb brain and inactivated with betapropiolactone.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>208</td>
<td>0</td>
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<td>212</td>
<td>0</td>
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<tr>
<td>216</td>
<td>0</td>
</tr>
</tbody>
</table>

a- Titers on day of challenge as indicated in Table XI.
TABLE IX

Group "H"
Serum Neutralizing Antibody Titers in Cattle vaccinated with
one dose of inactivated fixed virus vaccine made from
suckling mouse brains with the addition of Al(OH)$_3$

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Antibody Titer on Day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>221</td>
<td>0</td>
</tr>
<tr>
<td>223</td>
<td>0</td>
</tr>
<tr>
<td>224</td>
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<td>238</td>
<td>0</td>
</tr>
<tr>
<td>242</td>
<td>0</td>
</tr>
</tbody>
</table>

* Died four days after this bleeding from an intercurrent disease.

a- Titers on day of challenge as indicated in table XI.
b- Titers on day of challenge as indicated in table XII.
TABLE X
Reciprocal of Median Neutralizing Antibody Titers of Cattle Representing each Experimental Group.

<table>
<thead>
<tr>
<th>Group Vaccinated</th>
<th>No. of Cattle Vaccinated</th>
<th>Animals with detectable antibodies/Reciprocal of median titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Samples taken on day:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A (ERA)</td>
<td>8</td>
<td>0/0</td>
</tr>
<tr>
<td>B (ERA)</td>
<td>8</td>
<td>0/0</td>
</tr>
<tr>
<td>C (HEP-CEO)</td>
<td>20</td>
<td>0/0</td>
</tr>
<tr>
<td>D (HEP-CEO+A1)</td>
<td>8</td>
<td>0/0</td>
</tr>
<tr>
<td>E (HEP-DKC)</td>
<td>10</td>
<td>0/0</td>
</tr>
<tr>
<td>F (SMBV)</td>
<td>10</td>
<td>0/0</td>
</tr>
<tr>
<td>G (SLBV)</td>
<td>8</td>
<td>0/0</td>
</tr>
<tr>
<td>H (SMBV + A1)</td>
<td>10</td>
<td>0/0</td>
</tr>
</tbody>
</table>

* Groups A and F received booster dose on this day.
  a- These groups of animals have not reached this period.

TABLE XI
Results of Challenge of Cattle Vaccinated with Rabies Vaccine.

<table>
<thead>
<tr>
<th>Groups of vaccinated cattle (Type of vaccine)</th>
<th>Interval between vaccination and challenge in days</th>
<th>Median of antibodies titer on day of challenge</th>
<th>Challenge results Survivors/Challenge*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B (ERA)</td>
<td>900-930</td>
<td>31</td>
<td>5/5</td>
</tr>
<tr>
<td>C (HEP-CEO)</td>
<td>660</td>
<td>5</td>
<td>5/5</td>
</tr>
<tr>
<td>G (SLBV)</td>
<td>660</td>
<td>30</td>
<td>3/4</td>
</tr>
<tr>
<td>D (HEP-CEO+A1)</td>
<td>600</td>
<td>4 2</td>
<td>4/4</td>
</tr>
<tr>
<td>H (SMBV + A1)</td>
<td>330</td>
<td>11</td>
<td>4/4</td>
</tr>
<tr>
<td>E (HEP-DKC)</td>
<td>300</td>
<td>4 2</td>
<td>2/5</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0</td>
<td>1/5</td>
</tr>
</tbody>
</table>

* Dose of Virus used in this Challenge was 2.18 million mouse intracerebral LD50.
RABIES IMMUNITY IN VACCINATED CATTLE

TABLE XII
Results of Challenge of Cattle Vaccinated with Rabies Vaccine.

<table>
<thead>
<tr>
<th>Groups of vaccinated cattle</th>
<th>Interval between vaccination and challenge in days</th>
<th>Median of antibodies titer on day of challenge</th>
<th>Challenge results Survivors/Challenge*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B (EBA)</td>
<td>1020-1050</td>
<td>93</td>
<td>5/5</td>
</tr>
<tr>
<td>C (HEP-CEO)</td>
<td>810</td>
<td>&lt; 2</td>
<td>0/5</td>
</tr>
<tr>
<td>H (MV + A1)</td>
<td>480</td>
<td>121</td>
<td>5/5</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0</td>
<td>2/8</td>
</tr>
</tbody>
</table>

* Dose of virus used in this challenge was 10.9 million mouse intracerebral LD50.
REFERENCES


In 1968, 3613 laboratory-confirmed cases of rabies were reported to the National Communicable Disease Center; this is a 22 percent decrease from 1967 and an 18 percent decrease from the average for the last five years. Forty-seven states reported animal rabies; only Hawaii, Rhode Island and South Carolina reported no rabies cases in 1968. Guam, which reported 89 cases for 1967 had no cases in 1968. Kentucky reported the largest number of cases with 413. The species accounting for the majority of laboratory-confirmed cases in 1968 were skunks (35%), foxes (22%), cattle (10%), dogs (8%), bats (8%), cats (4%), raccoons (4%), and equines (2%). Only 296 cases of laboratory confirmed rabies in dogs were reported in the United States in 1968; this is a 28 percent decrease from 1967 and a 33 percent decrease from the average for the last five years. One human rabies death occurred in 1968; in October a 13 year old boy died in Kansas.

A total of 863,773 individual doses of human antirabies vaccine were produced and distributed by the two commercial manufacturers; state health departments in Illinois, Michigan and Texas also produced human antirabies vaccine. The number of doses of human rabies vaccine distributed was approximately 300,000 more than in 1967. Approximately 6,000,000 International Units of equine origin antirabies hyperimmune serum were distributed in 1968.

In 1968, rabies virus was detected in 24 different animal hosts in the United States. Wild species were responsible for 75 percent of the cases in animals.

A total of 910 cases of rabies were reported in eight species of domestic animals during 1968, a reduction of 468 cases from the 1967 total.

The human death mentioned is the first in two years which was acquired in the United States.

Our committee heard a presentation by Dr. Harold B. Hubbard, Pan American Health Organization, Washington, D.C. on "Rabies Immunity in Vaccinated Cattle". This paper will be printed in the 1970 U.S. Animal Health Association proceedings.

Last year we recommended that the National Research Council be asked to sponsor a National Rabies Advisory Council to develop a system of nationwide rabies control. The initial approach has been made and representatives of the committee are planning to attend the meeting of the National Research Council, Committee on Animal Health in December this year.

Active interest in an international rabies symposium has been indicated by the Pan American Health Organization and the World Health Organization. Suggestions were made that these organization jointly sponsor such a meeting and be responsible for printing the proceedings.

We are indebted to the National Communicable Disease Center for the statistical portion of this report and the maps and charts included.
NUMBER OF RABIES CASES REPORTED BY STATE - 1968

TOTAL CASES
LABORATORY CONFIRMED - 3,613
CLINICAL DIAGNOSIS - 126
EVALUATION OF THE TRICHINOSIS PILOT PROJECT

and N. E. Schulz, D.V.M., B.S.***

At the October, 1967, meeting of the United States Livestock Sanitary Association (USLSA), Dr. W. J. Zimmerman of Iowa State University, described a pooled sample technique for the post-slaughter detection of trichinae in swine. This technique was enthusiastically received by representatives of the pork industry as a possible tool for bringing about the ultimate eradication of trichinae from swine in the United States. This favorable attitude of the industry toward this technique led to the initiation, in July, 1968, of the Trichinosis Pilot Project at Hormel and Company, Fort Dodge, Iowa. This project was the result of the cooperative effort of the pork and allied industries (coordinated by Livestock Conservation, Incorporated) and the Agricultural Research Service and Consumer and Marketing Service of the United States Department of Agriculture.

The history and development of this project and the results of the first 10 weeks of full scale operation were reported to the USLSA at New Orleans in October, 1968. At that time, the project was scheduled to continue for a full year. However, in December 1968, it was decided to examine the results obtained up to that time with a view to determining the advisability of continuing the project for the full 52-week period.

At a meeting in Fort Dodge, Iowa, in January, 1969, the collaborators decided to terminate the project at the end of 32 weeks of full-time operation, with the understanding that tracing trichinous hogs back to their farm of origin and the collection of related epizootiologic data by the Animal Health Division, ARS, would continue through June 30, 1969.

The present report covers the 32-week period from July 22, 1968, through February 28, 1969, and includes descriptions of the laboratory, laboratory equipment and technique, personnel information, data on the pigs examined and efficiency of the technique, cost per head of the examination, and results to date from the epizootiologic study. The implications of the results as related to the possible development of an all-out national trichinosis eradication program are also discussed.

Materials and Methods

The trichinosis laboratory was located in a 28' by 15' room on the same level as the killing floor. Four incubators 30" deep, 76" high and 84" wide occupied one side of the room (Fig. 1). A 5th incubator and a work bench, which extended the remaining distance, were located along the opposite wall (Fig. 2). The top shelf of

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the first 4 incubators (Fig. 3) was designed to hold 36 3,000 ml. stainless steel beakers and was equipped with an electrically operated mechanical stirring apparatus to agitate the contents of the beakers during the digestion process. The second shelf supported 18 stainless steel funnels 7" in diameter, and a third shelf held 18 stainless steel funnels 5" in diameter. The 5th incubator was like the other 4 except that the 3 shelves within it were designed to hold 3,000-ml beakers and each was equipped with a stirring apparatus. The top shelf held 36, and the other two each held 33 beakers. Each incubator was heated by a 400-watt electrical heating element controlled by a thermostat.

A stainless steel double sink with double drainboards was located in the middle of the laboratory (Fig. 1). A graduated 150-gallon cylindrical Nalgene tank fitted with a faucet through which the contents could be withdrawn was located at the far end of the laboratory (Fig. 4). It was used for mixing and storing the digestive fluid used each day in the procedure. Water, maintained at a temperature of 120° F. by an in-line thermostat, was applied to the tank from a faucet located directly above it.

Other laboratory equipment included:
- 2 Heavy duty electrically operated meat choppers
- 50 Grinder heads (No. 10)
- 2 Plates for grinder heads (3/16")
- 2 Stereomicroscopes (Max. Mag. 70x)
- 2 Microscope lamps
- 1 Electric clock timer (24 hr.)
- 1 Balance (10 kg. cap.)
- 1 Dietary scale (500 gm. cap.)
- 250 Stainless steel beakers (3,000 ml cap.)
- 3 Laboratory carts
- 72 Stainless steel funnels (Dia. 7" with vertical sides to increase capacity to 3,000 ml)
- 140 Stainless steel screens to fit 7" funnels (80-mesh/inch)
- 72 Stainless steel funnels (Dia. 5", standard)
- 72 Hose clamps
- Thin-walled rubbed tubing (I.D. 1/2", and I.D. 3/8")
- Glass tubing (O.D. 3/8") (for siphons)
- 250 Plastic paddles (9" x 2½" x ¼") for stirring apparatus
- 100 Glass dishes, Dia. 2½", transparent, flat-bottomed, etched with ½ cm. squares
- 25,000 Paper cups (No. 10)
- 10 Aspirator bulbs (to start siphons)
- 2 Graduated cylinders (500 ml)

Chemicals:
- 10 Carboys (127 lb.) Hydrochloric acid (Concentrated)
- 417 lb. Pepsin (Granular 1/10,000)

Biological material:
- Trichinous meat for controls

Miscellaneous supplies:
- Assorted knives, knife sharpeners, 3- and 4-gallon pails, masking tape, wire
or wrought-iron carriers to transport paper cups to and from the kill floor, wax paper, paper towels, pencils for marking carcasses, beakers, and funnels used in the laboratory, laboratory stools, small step-stool, soap, detergents, forms for keeping appropriate records, and miscellaneous office supplies.

Personnel

Nine persons were directly employed on the project: 1 project leader (a veterinarian), 4 laboratory technicians, 2 laboratory helpers, and 2 employees who were assigned to the kill floor. The project leader was responsible for the overall conduct of the work and for reporting the results to the ARS Trichinosis Evaluation Group through the Technical Services Division, C&MS.

Two laboratory technicians and 1 helper were assigned to the day crew and the same number to the night crew. These crews alternated shifts on a 2-week basis.

The laboratory technicians mixed the digestive fluid, prepared the meat samples for digestion, processed and examined them for trichinae, and recorded and reported the results to the project leader.

The laboratory helpers labeled and delivered paper cups to the kill floor, carried meat samples back to the laboratory, ground the meat samples, washed and set up the various laboratory equipment, and assisted in overall laboratory maintenance.

The employees on the kill floor, assigned to the day crew, marked lot numbers on carcasses and collected diaphragm samples.

Preparation to receive specimens from the kill floor

Each morning the number of hogs to be killed that day was ascertained and the required volume of digestive fluid was prepared according to the following formula:

For each 300 5- to 8-gram diaphragm specimens:
- 10 gallons H₂O at 120°F.
- 125 grams 1/10,000 pepsin
- 380 ml. HCl

The water was supplied to the tank at 120°F. to eliminate the need for an auxiliary heater to maintain the digestive fluid near 100°F. during the 8- to 10-hour working day.

Pieces of masking tape were affixed to the paper cups into which would be placed the pooled samples of diaphragm muscle from each lot of 20 to 25 hogs. Lot numbers were then put on the masking tape. The first 18 cups were numbered A-1 through A-18, the second 18, B-1 through B-18, the third, C-1 through C-18, etc. The size of the series was determined by the number of pooled samples that could be processed in 1 incubator at 1 time. The number of series was determined by the total number of hogs slaughtered and examined each day.

Work on the kill floor

During the aforementioned period, the hogs that had passed the ante-mortem inspection were desensitized in the carbon dioxide immobilizer and exsanguinated. The carcasses were then scalded, dehaired, washed, eviscerated, inspected, and subjected to further processing to prepare them for overnight storage in the cooler at about 40°F.
After postmortem inspection, the carcasses were divided into lots of 20 to 25. Each group of carcasses was then marked on the hock with a lot number matching one put on the paper cups. (The number was located on the hock so that the carcasses could be readily located from a distance when hanging in the cooler). A 5-to 8-gram sample of muscle tissue excised from the diaphragm pillars of each carcass was dropped into the cup marked with the appropriate lot number. When 9 to 10 pooled samples had been collected, they were placed in a suitable carrier and transported 125 feet to the trichinosis laboratory.

**Preparation and Examination of Diaphragm Muscle for Trichinae**

Each lot of samples in its proper order was ground in a separate, clean meat chopper. The masking tape with the number was removed from the paper cup and attached to the stainless steel beaker into which the ground meat dropped from the grinder. The grinder head was detached from the power unit, washed, rinsed and dried for use with subsequent composite samples. A clean grinder head was then attached to the power unit and the next lot ground, the beaker identified by its number, and set alongside the first one.

When 18 lots had been ground and transferred to the beakers, 2,500 ml. of digestive fluid were added to each beaker. A plexiglass paddle was put in each one and the beakers were put on the top shelf of an incubator. The paddles were attached to the stirring mechanism, the mechanism started, and the time, 10 hours hence, when the mechanism was set to stop, was marked on a chart on the incubator door. One man-hour per day was saved and the subsequent operations speeded up 1 hour by setting the automatic timer to stop the stirring apparatus of the first incubator to be filled one hour before the night employees reported for duty. The above series of operations was repeated about once each hour until all diaphragm samples from the day’s kill had been put in the incubators.

The first 4 series of 18 pooled samples were placed in the 5th incubator, which was designed solely for digesting the specimens. After digestion was completed the contents of the beakers were transferred to 1 of the other incubators where the settling-out process was carried out. The next 4 series of 18 composite samples were put on the left side and the next four on the right side of the 4 incubators containing the funnels, respectively. These procedures permitted three sedimentations to be carried out in each of these incubators during the digestion of other specimens and minimized the possibility of mixing one group with those of adjacent ones by separating their processing by 4 hours. The remaining capacity of the 5th incubator was used for re-runs and for the digestion of specimens that could not otherwise be accommodated.

**Preparation of Controls**

Controls were prepared by adding 1 to 116 trichina cysts to each of 403 lots of known uninfected pork containing 20 to 25 five-to-eight-gram samples of meat. The trichinous meat used in the controls was obtained from the carcasses of rats or pigs that harbored infections at least 30 days old. The number of cysts added was determined by direct count of the cysts in press preparations of the muscle tissue under a standard dissecting microscope.

Calculations of the number of trichinae in each control lot were made as though all the trichinae that had been added were in one of the 5-to-8-gram
samples. The remaining samples in the lot were considered to have no trichinae. Thus, the addition of 5 trichinae per 100 grams of sample was considered the equivalent of one-trichina-per-gram of diaphragm from a single hog.

The control lots were inserted randomly in the lot series to monitor the efficiency of the technique and the alertness of the technician.

Post-digestion handling of specimens

When the last of the day's specimens had been put in the incubators, the laboratory was cleaned and made ready for the night crew which was responsible for completing the digestion and sedimentation of the specimens, examining them for trichinae, and reporting the results to the project leader. The night operation was necessary because the trichinous lots had to be identified and their lot numbers transmitted to the cutting room foreman by 6:30 the next morning.

When the night crew arrived, the first 18 lots had already settled for one hour and were ready for the next step in the procedure. About two-thirds of the supernatant was siphoned off and discarded taking care not to disturb the sediment in the bottom of the beakers. The remaining third of the contents was poured through the 80-mesh screens into the 7" funnels which were closed at the bottom with clamped rubber tubes. Identification of the specimens was maintained by transferring the masking tape with the lot number on it from the beaker to the appropriate funnel. The beakers were then rinsed with warm water which was also poured into the appropriate funnel. The funnels were then filled with warm water (110°F.) to a level just above the screen proper and the contents were allowed to remain undisturbed for one hour.

At the end of this period, the 5-inch funnels were filled by draining the lower portion of the contents of the 7-inch funnels into them. This permitted adding water to specimens containing an unusual quantity of debris to further clarify the sample for microscopic examination. The contents of these funnels were allowed to settle for one hour. Ten to 15 ml of sediment in each 5-inch funnel were then drawn into a flat-bottomed transparent glass dish for microscopic examination. Identification of the samples was maintained by placing the glass dishes in numbered circles painted on a board to correspond with the lot numbers of each series.

The technician required from one to three minutes to examine each lot at 25 to 30x magnification and to record the results and condition of the specimen, i.e., whether it was cloudy or clear or contained flocculent material or debris.

All trichinous lots were identified and the project leader was informed of the results of the examinations on his arrival at his office the next morning. He relayed this information to the cutting room foreman who held the "suspect" lots in the cooler for re-examination. "U.S.D.A. Retained" tags were put on all carcasses in these suspect lots.

As soon as the suspect lots were in a position in the cooler where they could be examined conveniently, a 37- to 144-gram sample of diaphragm muscle was excised from each carcass and identified by the retained tag number. Each sample was then processed individually and examined for trichinae in the same manner as were the composite samples on the previous day, except that a smaller volume of digestive fluid was used.
After the trichinous carcass or carcasses within a suspect lot were identified, the nontrichinous ones were immediately released to the cutting room.* The trichinous carcasses were held under supervision until they had been treated according to the procedures prescribed by Federal regulations to destroy trichinae.

Tattoo numbers of infected carcasses were recorded and forwarded to the livestock buying department of Hormel and Company which provided names and addresses of the owners or dealers from whom the hogs were purchased, the live weights of the hogs, and the purchase date. This information was sent to the U.S.D.A.'s Animal Health Division Office, Des Moines, Iowa, for use in tracing the trichinous hogs back to the farm of origin. Epizootiological investigations were carried out by State and Federal regulatory veterinarians with a view to preventing recurrence or spread of the infection.

**Results**

A total of 482,392 hogs was examined during the 32-week period the project was in full operation (Table 1). Of these, 42 hogs were trichinous. The number of hogs examined each week and the distribution of the trichinous hogs detected by the project are recorded graphically in Fig. 5.

The data in Fig. 5 show that from 9,108 to 19,484 hogs were examined each week and that from 1 to 10 trichinous hogs were detected in 16 of the 32 weeks. The data also show that trichinous hogs were scattered throughout the period. The larger number of infected hogs identified during the 2nd, 9th, and 28th weeks were the result of 3 farms contributing more than 1 trichinous hog to the project (see Epizootiology).

The infection levels of the 42 trichinous hogs detected by the project are indicated in Fig. 6. Sixteen (38%) with infections less than 0.01 trichina per gram of diaphragm muscle were detected only in the composite sample. Twenty-six were identified. Eight (19% of the total) had infections of less than 1 trichina per gram, and 18 (43% of the total) had infections greater than 1 trichina per gram.

The number of trichinous hogs, the incidence of infection for each weight class and for the total number examined are summarized in Table 1. These data show that the overall incidence of trichinae was very low, but that the older and heavier hogs were more likely to be trichinous than the younger market hogs. The infection rates of the hogs weighing 400 pound or more and those weighing from 300 to 400 pounds were 5 and 1.8 times that of the entire group, respectively. The infection rate of the 180 to 300 pound class was 0.7 of the average incidence of the entire group.

The relationship between weight (and age) and the intensity of infection is shown in Table 2.

The data in Table 2 show that 24 of the 33 very light and relatively light infections occurred in hogs weighing 180 to 300 pounds; whereas, 6 of the 9 moderate to heavy infections occurred in hogs weighing 400 pounds or more.

**Comparison of the digestion and trichinoscopic methods of diagnosis**

Twenty-two of the 26 samples of trichinous pork obtained from identifiable

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* For the purposes of this project the head meat was not exempted from regulatory controls.
swine with infections ranging from 0.01 to 641.7 trichinae per gram of diaphragm tissues were examined by the digestion method and the trichinoscopic method in order to compare the two techniques. Diaphragm samples weighing from 37 to 144 grams were taken from individual hogs and digested as indicated earlier in this paper. Samples of the same tissue each weighing approximately one gram were examined as press preparations with the trichinoscope. The results are recorded in Table 3.

The data in Table 3 show that trichinae were detected by both methods in those samples having 5.8 trichinae or more per gram of tissue, but that the trichinoscopic method failed to detect them in samples having 5.6 or fewer trichinae per gram. The data also show that the number of cysts detected by the trichinoscope was always less than the number detected by the digestion method and that the number observed with the trichinoscope was not always consistent with the numbers observed by the digestion technique.

**Controls**

The extreme sensitivity of the pooled sample digestion technique is shown by the following results:

When one or more cysts in trichinous rat meat, which had been held at 40°C, 1 to 5 days after the death of the host, were added to 100-gram quantities of trichina-free pork, at least one trichina was recovered from 29 of 30 lots (97%). When less than one trichina per gram, 1 to 4 days old, was added, at least one trichina was recovered from 11 of 16 lots (69%).

In another series of 66 control lots to which weighed quantities of trichinous pork with known average numbers of trichinae per gram were added, the residues of 33 and 35 lots (94%), to which 1 to 2 trichinae per gram had been added, contained trichinae. More significantly, the residues of 30 of 31 lots (97%) to which less than 1 trichina per gram had been added, contained trichinae. Since the number of cysts added to the controls of this series was based on the average numbers of trichinae per gram observed in the infected meat, it is possible that the three negative lots may not have contained cysts.

The results obtained from the examination of 343 control lots containing from 0.01 to 0.22 trichinae per gram of ground meat (0.2 to 4.4 per gram of each 5-gram diaphragm sample) are recorded in Fig. 7. These data were compiled without taking into account the period the trichinous meat was held at 40°C. following the death of the host.

Although the over-all sensitivity of the test, as indicated by the data in Fig. 7, may have been reduced by the poor initial condition of some of the trichinae used in the tests, and by other factors to be mentioned later, the data show that trichinae were detected in 60 percent of the trials when .01 trichina per gram of composite sample was present (0.2 trichinae per gram of 5-gram sample), in 88 percent, when .05 trichinae per gram was present (1 trichina per gram of 5-gram sample), and in 100 percent, when .12, .17, and .22 trichinae per gram (2.8, 3.4, and 4.4 trichinae-per gram of 5-gram sample) were present.

Data on the 60 trials not graphed demonstrated that trichinae were detected at all 28 control levels from .23 to 1.16 trichinae per gram of composite sample (4.6 to 23.2 trichinae per gram of 5-gram sample), except one. In this instance, the
trichinous meat had been exposed to 40°C for 16 days prior to the test and may not have survived digestion.

**Effect of storing trichinous meat at 40°C on recovery of trichinae from controls**

Since it was not always possible to use fresh trichinous meat as the source of trichinae for the control samples, data were obtained on the effect of storing it at 40°C for various periods after the death of the host on the recovery of larvae from the digested meat. The data from 322 tests in which meat containing 32 to 850 (Avg. 118) encysted trichinae was added to trichina-free pork samples are recorded in Fig. 8.

The data in Fig. 8 show that the percentage of larvae recovered after digestion of the sample in general decreased as the length of the period between necropsy of the host and the test day increased beyond 5 days. This result was attributed to the progressive inability of the larvae to withstand digestion after prolonged periods of exposure to 40°C.

Some factors that may have influenced the failure to find trichinae in all of the control samples tested, and failure of the data to produce a smooth time-survival curve were (1) differences in the condition of the larvae at the time they were subjected to digestion, (2) alertness of the technician who examined the samples, (3) and condition of the sample being examined, which may have interfered with the observation of the parasites.

**Cost of Test**

The total cost of the pooled sample digestion technique for the post-slaughter examination of hog carcasses for trichinae at the Hormel and Company plant, Fort Dodge, Iowa, was calculated to be $51,874.02. The cost per-head of examining the 482,392 hogs during the 32-week period if recorded in Table 4.

The data in Table 4 show that the overall cost per hog examined was 9.35 cents. This is about 0.067 cents per pound of dressed pork, when the cost is calculated in the basis of a 70% yield from a 200-pound hog.

In a previous calculation, one-tenth of the supervisor's salary was included in the total labor charge because he would be required to supervise 10 laboratories in an expanded program. One-fourth of the project leader's salary was included in the present figure to provide money for the hiring of higher salaried technicians who would be required to supervise the work in each of the proposed 10 laboratories.

**Epizootiology**

Efforts were made to trace back to the farm of origin the 26 trichinous hogs that were identified by the project. In each instance, more than 1 herd was usually implicated as a possible source of the hog in question. Three swine dealers were involved in the course of the investigation. One dealer had purchased hogs from 10 different owners, 1 from 7, and 1 from 2. Occasionally, the herd owner had acquired all or a portion of his swine from several sources, thus incriminating the

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* Funds for labor and equipment were provided by the Animal Disease and Parasite Research Division, ARS, and the Animal Health Division, ARS. The Consumer Protection Programs, C&MS, administered and carried out the procedures within the packing house and furnished the salary of one laboratory technician. (Also see acknowledgements).
original herds as possible sources of trichinae. In all, more than 40 herds were suspect and were considered to be potential sources of the trichinous carcasses. The epizootiological studies indicated that of the suspect herds, 9 were most likely to be sources of the trichinous swine. Four of these had a history of exposure to raw garbage and 2 had been fed carcasses of wild mammals. Trichinae were found in a fox diaphragm from 1 farm on which the owner had fed carcasses of fur bearing animals to swine that were identified as trichinous. The other 3 herds were the primary sources of the trichinous hogs originating from them, but conclusive evidence of exposure to trichinae could not be ascertained. Six trichinous hogs originated on 1 farm, 3 on another and 2 herd owners contributed trichinous carcasses to the project on 2 separate occasions. These findings indicate that a premise on which conditions are favorable for trichina transmission may be the source of more than one trichinous pig during 1 grazing season. The elimination of feeding practices responsible for these infections would result in the disappearance of these sources and thereby contribute to the eventual eradication of trichinosis.

Evidence of infection was inconclusive in the remaining 30 or more suspect herds. On many occasions attempts to find the source of infection revealed that the herds in question had been fed table scraps. For others, potential sources of trichina infection were not found.

In each investigation, regulatory personnel made every attempt to discuss trichinosis with the herd owner by describing the disease and offering information on mode of transmission and control. They also suggested sanitation and management practices that would alleviate or prevent herd infection.

Benefits
Benefits accruing to the processor through the use of this method are of two kinds: (1) cost reduction and (2) increased production. The first could be realized by the elimination of the need to freeze or otherwise process pork to destroy trichinae, as well as costs directly associated with the inspection of products undergoing special processing for the destruction of trichinae. The second would be realized through shortening the processing time now required for all products intended to be eaten without further cooking to assure the destruction of trichinae. The more rapid turnover of these products would enable a plant to obtain greater production from the same equipment without a major increase in overhead expense.

Benefits to the swine producer would come about indirectly. Assurance that all pork had been examined for trichinae would undoubtedly remove this stigma now associated with pork, thereby increasing demand for pork, both at home and abroad.

Future of Pooled Sample Technique
At the present time, the Consumer Protection Programs, C&MS, is in the process of utilizing this method for the examination of hams and shoulders intended for salt-dry curing. This will be done in lieu of destroying trichinae by conventional methods and certifying that the products have been subjected to long-term time and temperature controls for the destruction of the parasite. Examination of these cuts at a central laboratory eliminates on-site inspection at
the various plants during the curing process, thereby reducing the cost of this service.

A joint plan for the eradication of trichinae from swine is being presented to the Secretary of Agriculture which would utilize the pooled sample technique. Such a program if adopted on a national scale, will do much toward eliminating trichinosis.

Discussion

The pooled sample digestion technique for the post-slaughter examination of hog carcasses for trichinae is the first such technique to be developed for use in high speed slaughtering operations. This project demonstrated that it could be successfully adapted to a commercial hog slaughtering plant operating at a rate of 460 hogs per hour (3,680 hogs per 8-hour day) without disturbing the work flow. By appropriate modification it could easily be adapted to a much faster operation than this. The data obtained confirm preliminary laboratory findings that the pooled sample technique was effective in detecting trichina infections as small as one larva per 100 grams of pork (0.2 trichinae per gram of one 5-gram diaphragm sample). Thus, this method exceeds the sensitivity that would be required to detect an infection of one trichina per gram of diaphragm muscle of any one hog in a 20- to 25-hog composite lot sample: the minimal limit of sensitivity (arbitrarily set) that would have to be met by any practical method of trichina detection.

The subsequent failure of the method to detect trichinae in individual pigs examined from 16 composite samples in which only one trichina had been found was interpreted to mean that the infection present in the infected pig was smaller than could be detected in the 37- to 144-gram diaphragm sample examined on the retest.

The incidence of infection of the hogs examined during this project (0.0087%) was very low as compared to the 0.12% found in butcher hogs of the country as a whole. This can be accounted for by the very high standards of management and feeding practices followed by the majority of producers whose hogs were examined at Fort Dodge.

The finding that trichinae occurred more frequently and in larger numbers in the older and heavier hogs than in the young market hogs indicated that the older hogs had probably had greater exposure to trichinae than the market hogs.

The per-head cost of 9.35 cents for the examination of hogs for trichinae by this technique was found to be about one-fifth of the cost of a trichinoscopic examination, which was estimated to be 46 cents per hog. The pooled sample technique was carried out with 8 employees which was one-seventh of the 56 persons that would be required to examine the same number of hogs with the trichinoscope. However, the latter advantage would be increased to about one-sixth in actual practice because the cost of pepsin and acid would be about equal to the salaries of 2.5 employees.

This is reason to believe that the present cost can be substantially reduced by (1) mechanizing or automating portions of the procedure, such as the formulation and mixing of the digestive fluid, transporting cups and composite meat samples between the laboratory and kill floor, moving beakers containing the samples from the grinding area to the incubators, and adding digestive fluid; (2) reducing the time
required to digest the meat samples; (3) including more diaphragm samples in each lot digested; (4) using less pepsin per 100 grams of meat than was used in the pilot project; and (5) working at the full capacity of the laboratory. The pilot project could have handled 90,000 to 95,000 additional hogs during the 32-week period without any increase in the labor force if the meat packing plant had operated at its full capacity each day.

One of the important advantages of conducting the trichinosis pilot project in Iowa was the opportunity afforded by the state hog identification system which enabled the Animal Health Division to trace trichinous hogs back to the farm of origin and to conduct epizootiologic studies on the infected premises. This study revealed an unsuspected relationship between the intentional feeding of carcasses of fur bearing mammals to swine and the occurrence of trichinae in the latter. Fur bearing mammal carcass feeding to swine was involved in 22 percent of the 9 trichinous herds investigated and may indicate that this is an important source of trichina infection in grain fed hogs. Little or nothing is known of the natural transfer of trichinae from sylvatic animals to swine, however, this is not considered to be an important natural source of the parasite. The other sources of trichinae, raw garbage and table scrape, noted in the investigation are also important sources of trichinae when fed to swine or any other susceptible animal. All of these sources can be eliminated by observing proper sanitary procedures in feeding and management of swine.

The finding that 1 farm was the source of 6, 1 for 3, and 2 for 2 trichinous hogs, indicated that premises on which conditions for the transmission of trichinae are present can be the source of more than 1 infected hog in 1 grazing season. The elimination of such sources would also work toward the ultimate eradication of trichinae from swine in the United States.

It would appear that the chief savings derived from the use of this method would accrue to the slaughterer who manufactured his own pork products. He would be relieved of the cost of freezing pork intended for products intended to be eaten without further cooking, and of inspection costs for the processing of these products to insure their being heated sufficiently or otherwise treated to destroy trichinae that might be present in the meat.

The separation of the head and loss of identity of the head meat of carcasses on the kill floor before the latter were inspected for trichinae was the only serious flaw in the operation of the project. This situation could be corrected by leaving the head on the carcass until after its status was determined.

Despite a few imperfections revealed by the trichinosis pilot project, the pooled sample digestion technique is still considered to be a significant step forward in the effort to bring about the eradication of trichinae from swine in the United States. Now that certain imperfections are recognized, it is believed that their effects can be minimized or eliminated in future applications of the technique in other plants.

Summary
1. A pooled sample digestion technique for the post-slaughter detection of trichinae in hog carcasses was tested for 32-weeks in a commercial meat packing establishment and was found adaptable to a slaughter speed of 460 hogs per hour
without disturbing the workflow (3,680/8 hr. day).

2. Forty-two of the 482,392 hogs examined (0.0087%) were trichinous. The incidence of infection in 180- to 300-pound market hogs was 0.0064%, in 300- to 400-pound hogs, 0.0159%, and in hogs weighing 400 pounds or more, 0.0433%.

3. The intensity of infection ranged from less than 0.01 to 641.7 trichinae per gram of diaphragm muscle, and was usually greater in the older and heavier hogs. Sixteen (38%) with infections of less than 0.01 trichina per gram of diaphragm muscle were detected only in the composite samples. Eight (19%) had infections of less than one trichina per gram, and 18 (43%) had infections greater than one trichina per gram.

4. The digestion method was shown to be more sensitive and cheaper than the trichoscopic method for the detection of trichinae.

5. Examination of controls to which counted numbers of encysted trichinae in meat were added to trichinae-free pork demonstrated that the pooled sample technique detected infections as small as 1 trichina per 100 grams of composite sample (0.2 trichinae per gram of one 5-gram diaphragm sample). It could be depended upon to detect as few as 5 trichinae per 100 grams of composite sample (1 trichina per gram of one 5-gram diaphragm sample) in about 97 percent of the trials.

6. Encysted trichinae in meat used for the controls could not be depended upon to survive the digestive process after being stored more than 5 days at 4°C after the death of the host.

7. The per-head cost of examining the hogs for trichinae was 9.35 cents. (0.067 cents per pound of dressed pork).

8. Nine of the 26 hogs, identified as trichinous, were traced back to their farm of origin. Epizootiological investigations revealed that raw garbage, carcasses of wild mammals, and table scraps were the chief sources of the trichinae in these hogs. Farms on which conditions were favorable for the transmission of trichinae were the sources of more than one trichinous hog during the same grazing season.

9. This study demonstrated that the pooled sample digestion technique is a practical, economical tool to detect trichinae in slaughtered swine.

10. The evidence reported herein supports the initial belief that this technique can be adapted for use in a nationwide trichinosis eradication program that can achieve that goal in the foreseeable future.

Acknowledgements

Grateful acknowledgement is made to the following organizations, without whose cooperation this project could not have been carried out: Hormel and Company, Austin, Minnesota, and Fort Dodge, Iowa, which arranged to house the project, Cudahy Laboratories, Omaha, Nebraska and Wilson Laboratories, Chicago, Illinois, which furnished the pepsin, National Livestock and Meat Board, Chicago, Illinois, and National Pork Producer's Council, Des Moines, Iowa, which lent financial support, and Livestock Conservation, Incorporated, Hinsdale, Illinois, which coordinated the industry effort.

Also to Dr. Frank N. Hughes, C&MS, Officer-in-Charge of the Trichinosis Pilot Project, Dr. W. J. Zimmerman, Iowa State University, who gave freely of his services during the design and installation of the laboratory equipment and
contributed trichinous meat for the control samples, and to numerous unnamed employees of Hormel and Company who contributed ideas and designed special equipment to facilitate the smooth operation of the laboratory.
Fig. 1. View of trichinosis laboratory showing centrally located sink and incubators along the west wall.

Fig. 2. East side of laboratory showing work bench and fifth incubator.
Fig. 3. Inside view of one of the four incubators used for the digestion and sedimentation of the diaphragm samples.

Fig. 4. Storage tank for digestive fluid and fifth incubator used only for digesting specimens.
Fig. 5. Number of hogs examined weekly and number and distribution of trichinous hogs detected by the pooled sample technique.
Fig. 6. Infection levels of trichinous hogs detected by the pooled sample technique.
Fig. 7. Percentage of trichinae recovered from lightly infected controls.
Fig. 8. Effect of storage of trichinous meat at 4°C on recovery of trichinae from controls.
TABLE 1. INFECTION RATES OF HOGS ACCORDING TO WEIGHT CLASS

<table>
<thead>
<tr>
<th>WEIGHT CLASS</th>
<th>HOGS EXAMINED (Number)</th>
<th>TRICHINOUS HOGS (Number)</th>
<th>INFECTION RATE (Number-Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180-300</td>
<td>429,453</td>
<td>26</td>
<td>1/16,517 0.0064</td>
</tr>
<tr>
<td>300-400</td>
<td>25,214</td>
<td>4</td>
<td>1/6,303 0.0159</td>
</tr>
<tr>
<td>400-Up</td>
<td>27,728</td>
<td>12</td>
<td>1/2,311 0.0433</td>
</tr>
<tr>
<td>TOTAL</td>
<td>482,392</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>AVERAGE</td>
<td></td>
<td></td>
<td>1/11,485 0.0087</td>
</tr>
</tbody>
</table>

TABLE 2. INTENSITY OF INFECTION ACCORDING TO WEIGHT CLASS

<table>
<thead>
<tr>
<th>TRICHINAE/GRAM</th>
<th>WEIGHT CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180-300 lbs.</td>
</tr>
<tr>
<td>Less than 1</td>
<td>17</td>
</tr>
<tr>
<td>1 - 9</td>
<td>7</td>
</tr>
<tr>
<td>17 - 25</td>
<td>2</td>
</tr>
<tr>
<td>99 - 180</td>
<td>1</td>
</tr>
<tr>
<td>229 - 642</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>27</td>
</tr>
</tbody>
</table>

TABLE 3. NUMBER OF TRICHINAE DETECTED PER GRAM IN COMPARABLE PORK SAMPLES FROM THE SAME CARCASS BY THE DIGESTION AND TRICHINOSCOPIC TECHNIQUES

<table>
<thead>
<tr>
<th>TEST NO.</th>
<th>POOLED SAMPLE DIGESTION TECHNIQUE</th>
<th>TRICHINOSCOPE TECHNIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>641.7</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>229.1</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>179.2</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>151.1</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>98.7</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>24.3</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>21.4</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>17.4</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>9.0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>5.8</td>
<td>2</td>
</tr>
<tr>
<td>11-22</td>
<td>5.6-0.01</td>
<td>0</td>
</tr>
<tr>
<td>ITEM</td>
<td>CENTS</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>SALARY (Labor Plus ¼ of Supervisor’s Salary)</td>
<td>6.685</td>
<td></td>
</tr>
<tr>
<td>PEPSIN</td>
<td>1.400</td>
<td></td>
</tr>
<tr>
<td>LOSS OF PRODUCT (Meat Tested)</td>
<td>0.354</td>
<td></td>
</tr>
<tr>
<td>SPECIAL PROCESSING (Labor, Freezing Trichinous Carcasses)</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td>RENT AND UTILITIES</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td>EXPENDABLE SUPPLIES (Including HCl, Soap, Towels, Etc.)</td>
<td>0.195</td>
<td></td>
</tr>
<tr>
<td>OPERATING COST</td>
<td>8.938</td>
<td></td>
</tr>
<tr>
<td>EQUIPMENT AND INSTALLATION (Amortized over a 10-year Period)</td>
<td>0.412</td>
<td></td>
</tr>
<tr>
<td>TOTAL EXPENSE PER HEAD</td>
<td>9.350</td>
<td></td>
</tr>
</tbody>
</table>
LITERATURE CITED


COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

United States Animal Health Association
Report for 1969
Milwaukee, Wisconsin – October 1969

Chairman: D. P. Gustafson, West Lafayette, Indiana
R. A. Bankowski, Davis, California; J. J. Barta, Chicago, Ill.; E. H. Bohl, Wooster, Ohio; H. W. Dunne, University Park, Pennsylvania; E. M. Dwyer, Boston, Massachusetts; J. E. Fox, Ashland, Ohio; D. A. Fuller, Des Moines, Iowa; R. E. Hall, Madison, Wisconsin; E. O. Haelterman, West Lafayette, Indiana; J. B. Nance, Alamo, Tennessee; E. I. Pilchard, Washington, D.C.; M. Ristic, Urbana, Illinois; and N. E. Schulz, Hyattsville, Maryland.

The Subcommittee on Criteria for Eradicable Diseases of Swine presented a report. It was approved and is appended to the report of this the parent committee. The subcommittee was asked to continue its work which, it is estimated, will be completed in two or three more yearly sessions of the association.

The Subcommittee on Transmissible Gastroenteritis presented its report. It was approved and is appended to this report. The Committee on Transmissible Diseases of Swine reaffirms the statement concerning the use of virulent TGE virus in pregnant swine to prevent the disease in baby pigs made in the report of the Subcommittee in 1968. The statement is as follows:

"It has come to the attention of this Subcommittee that there is an increased interest in the use of live virulent TGE virus for "vaccinating" pregnant swine. This "planned infection" procedure is accomplished by the oral infection of swine at least three weeks prior to expected farrowing, and is designed to provide passive immunity to the suckling progeny of the immunized sows during the critical period of the first few weeks of life. Although known to be effective, in providing immunity, this procedure has certain inherent dangers, such as: (A) infection will be established from which the disease might be unintentionally disseminated to other herds and, in doing so, cause considerable harm, (B) pathogens other than the TGE virus might be transmitted unknowingly, (C) persistent or enzootic TGE could be established, especially in those herds on a frequent or continuous farrowing program."

Progress in the evaluation of the pooled-sample method of diagnosis of trichinosis in swine was reviewed by Dr. John S. Andrews of the USDA. A separate report evaluating the results of the pilot project conducted at the Hormel Packing Company plant in Fort Dodge, Iowa was presented in the general sessions of the Association as a project report from this Committee. The Committee was impressed with the success of the project and wishes to draw attention to this splendid achievement made possible through cooperation between industry and public funds at Iowa State University under the direction of Dr. W. J. Zimmerman. Discussion of the project revealed that other facets are being explored. Investigations of trichinae infections have revealed that carcasses of wildlife fed to swine have been sources of infection. Methods of detecting trichinae infection in live swine are being sought. The Consumer Protection and Marketing Service of the USDA plan to apply the
pooled-sample method to dry salt-cured pork as the next trial of its usefulness to help in the effort to effect practical eradication of trichinae from U.S. swine. Dr. D. P. Gustafson, having been asked to represent the USAHA on the newly formed National Committee on Trichinosis sponsored by Livestock Conservation Inc., attended the first meeting of that Committee at O'Hare Airport in Illinois on July 17, 1969. In the first meeting the problem was reviewed, the pooled-sample method pilot test effort report was presented, and organizational plans were developed for the promotion of the use of the test to help in the trichinosis control program.

Mr. J. Marvin Garner, Executive Vice-President of the National Pork Producers Council described a survey of swine production problems which that organization completed during 1969. While much of the survey was concerned with non-disease oriented phases of production considerable emphasis was placed on seeking information on health problems of high priority to producers. He reviewed the plans of the Council to support research in areas of concern to producers.

Dr. D. A. Fuller, Director of Technical Services of Diamond Laboratories, Inc., presented the results of a survey concerning dysentery in swine which is associated with Vibrio coli. Information obtained from eight state animal disease diagnostic laboratories in the midwest indicate an increase in the prevalence of vibronic swine dysentery. Responses from thirty-seven (37) practicing veterinarians also reflected an increase in diarrhea of swine that clinically were reminiscent of vibronic dysentery. One large feeder pig marketing enterprise has found that though there seems to be an increase in swine dysentery it can be avoided by careful operation. Another indicated their recognition of the increased problem in the past few years. The treatment and control of the dysentery has become more difficult. We therefore present that the syndrome is of sufficient proportions to warrant the concern of regulatory officials. Methods of control must be sought. The attention of research groups is necessary for the determination of the cause and the development of effective prophylactic and therapeutic agents.
# REPORT OF COMMITTEE

## CHART I

<table>
<thead>
<tr>
<th>STATE DIAGNOSTIC LABORATORIES</th>
<th>NUMBER OF VIBRIONIC DYSENTERY DIAGNOSES (Drove Basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Dakota</td>
<td>35</td>
</tr>
<tr>
<td>Minnesota</td>
<td>2 3 12</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>15 24 46</td>
</tr>
<tr>
<td>Missouri</td>
<td>2 15</td>
</tr>
<tr>
<td>Illinois</td>
<td>3 4 5</td>
</tr>
<tr>
<td>Indiana</td>
<td>6 (1659 head) 16 (1650 head) 18 (6053 head)</td>
</tr>
<tr>
<td>Ohio</td>
<td>13 ( 200 head) 17 ( 185 head) 30 ( 550 head)</td>
</tr>
</tbody>
</table>

*September through December

**January through April

## CHART II

<table>
<thead>
<tr>
<th>STATE</th>
<th>NO. OF D.V.M.'S</th>
<th>INCREASE</th>
<th>DECREASE</th>
<th>NO CHANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>11</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>3</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>5</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td>2</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>6</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>24</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>
The term eradication was defined as, “The successful elimination of a disease producing agent from an animal population in a given geographical area.” The sub-committee submits the following four general criteria as those which may be used for selecting candidate diseases for eradication:

1. **The disease is of economic or public health significance.**
   (a) Public interest in eradication arises from the extent of economic loss or from the danger to public health. The economic loss is made up of the following factors:
   1. Direct cost to the producer in animal losses.
   2. Indirect and direct cost to consumer.
   3. Loss in international markets.
   4. Loss in public acceptance of products is a loss borne by the industry.

2. **Means of diagnosis are available.**
   (a) The means of diagnosis must be such as can be widely accomplished.
   (b) The cost of diagnosis must not be excessive in time, talent, or materials.

3. **Practical methods are available to eradicate the disease.**
   (a) Surveillance, quarantine, and/or test and slaughter methods should be possible with minimal distress to the producer.
   (b) Biological approaches such as vaccination, vector control, or sterilization programs should be applicable in some effective measure.
   (c) Chemical and/or physical methods should be possible to an effective degree such as the use of antibiotics, insecticides, heat treatment of important items, or the use of disinfectants.

4. **There are methods available to prevent re-entrance of the disease into the geographical area.**
   (a) Effective regulatory control measure must be available through legislation or directives.
   (b) Geographical or climatic barriers of strength are present in some cases to assist in prevention of re-entry.
   (c) International cooperation through mutual interest in the problem should be possible and sought if it is apropos.

(Executive Committee has not reviewed this report.)
REPORT OF COMMITTEE

REPORT OF THE SUB-COMMITTEE* ON
TRANSMISSIBLE GASTROENTERITIS OF SWINE

Chairman: E. H. Bohl, Wooster, Ohio

Previous reports1,2,3 of this Sub-Committee have given particular attention to a review of and comments on methods of diagnosis of transmissible gastroenteritis (TGE), with brief remarks on etiology, pathogenesis, vaccination and research needs. The sub-committee feels that further comments on "etiology" and "vaccination" are indicated because of some of the controversies that have surrounded these topics.

Etiology

Only one serologic type of virus has been described as causing TGE. This virus has many of the characteristics of the newly-suggested Corona group4 of viruses, such as: RNA containing, medium sized, moderately pleomorphic, bearing pear-shaped surface projections, and ether or chloroform labile.

There are several reasons suggesting this virus as the etiologic agent in TGE: (1) It is isolated in cell cultures from the intestinal tract of piglets having the typical clinical signs of TGE, although during early cell culture passage the cytopathic effect (CPE) is usually mild and transient. (2) Clinical signs of TGE can be produced in pigs by the oral administration of the early-passaged, cell-cultured virus. (3) Antibodies against this virus are produced by swine that have recovered from field outbreaks.

However, there has been hesitancy by some to accept this virus as a cause of TGE, and 2 reasons have been offered. (1) Some workers have isolated a virus from field cases which replicated but did not produce a CPE in cell cultures, even after several passages. Thus, these individuals have been reluctant to accept the above mentioned CPE virus as the causative agent. The present consensus is that the so-called non-CPE virus is of the same serologic type as the CPE virus and that the former will produce CPE when appropriate cell culture methods are used; such as the use of agar overlay technique, swine testicular cells, or swine thyroid cells. (2) A second reason relates to the poor results which have been encountered by some when the live high-passaged, attenuated, CPE virus is used as an immunizing agent, especially on pregnant swine for the purpose of providing passive immunity to their suckling progeny. This is in contrast to immunity which usually occurs following natural or artificial infection with virulent virus. There may be reasons to account for these results other than on the basis that the CPE virus is not the true cause of TGE. These include: (a) Inadequate levels of neutralizing antibody in the colostrum and, especially, milk of sows infected or vaccinated with the attenuated virus. Even more important might be the level of certain classes of immunoglobulins — especially of the secretory type — in the colostrum and milk. (b) Antigenic changes in the

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* This is a sub-committee of the Committee on Transmissible Diseases of Swine.
high-passaged, cell-cultured virus may have occurred resulting in the production of antibodies which are ineffective in the gut for neutralizing virulent virus.

**Vaccination**

A licensed commercial vaccine for TGE is not presently available and none appears to be in the immediate offing. A considerable amount of research on the development of vaccines has been conducted by state and federal research institutions and by veterinary biologic companies. Difficulties and delays have been encountered. The reasons given have been: (1) Inability of inactivated or live attenuated vaccines to provide adequate protection of neonatal pigs, and (2) Inability of some biologic companies to provide sufficient experimental and field data to meet licensing standards for commercial use.

The type of immunity or protection required in this disease is unique. The primary object is to protect the epithelial cells of the small intestine against infection and destruction by TGE virus, especially in very young pigs in which the mortality can approach 100%. At present, this method of passive immunity can be accomplished only when pigs receive TGE antibodies orally and rather frequently, as is provided when they nurse an immune sow. There is no other known disease in animals or man which requires that protection be provided in this manner. Likewise, there is no other viral vaccine which has been developed for this particular purpose. Nor has there been a detailed explanation to account for the mechanism of this type of immunity, except that antibodies in colostrum and milk of recovered sows will tend to protect the epithelial cells of the small intestine. However, recent information on the role of certain classes of immunoglobulins, especially those of the secretory type, may hold the key. Thus, the problems posed, both for the development and evaluation of such a vaccine, are of a new order, and partially account for the difficulties and delays encountered. Much more research is indicated so that these problems can be resolved. For example, of immediate importance is the development of a standard procedure to evaluate the passive immunity of pigs which are nursing vaccinated sows. A sub-committee of the North Central Regional Research Committee in Enteric Diseases of Swine is presently working to the development of such a procedure.
REFERENCES

THE FILTER PAPER DISC TECHNIQUE FOR THE DETECTION OF ANTIBODIES AGAINST HOG CHOLERA VIRUS

by
E. A. Carbrey, VMD, MS; N. H. Hesse, DVM; W. C. Stewart, DVM, MS;
J. I. Kresse, BS; E. E. Everson, DVM

From the Virology Section (Carbrey, Stewart and Kresse) Diagnostic Services, Animal Health Division, National Animal Disease Laboratory, P.O. Box 70, Ames, Iowa, 50010. Dr. Hesse is a Veterinary Medical Officer, ANH, 877 Federal Building, 210 Walnut Street, Des Moines, Iowa, 50309, and Dr. Everson is a Veterinary Medical Officer, ANH, Route 6, Sparta, Tennessee, 38583.

Appreciation is expressed to Mr. Michael Snyder for technical assistance, to Mr. G. D. Booth for the statistical analysis, and to personnel of Diagnostic Service, Animal Health Division for consultation.

Summary
The fluorescent antibody serum neutralization test employed to detect antibodies against hog cholera (HG) virus has been adapted for use with swine blood samples collected on filter paper discs, 30 mm in diameter and 1.5 mm thick. Approximately 1.1 ml. of blood collected from an ear vein can be dried on the paper disc for convenient storage and shipment to the laboratory. The technique was standardized and good correlation was obtained on 162 replicate specimens, serums and blood discs from the same pig, from 29 swine herds where HC infection was suspected. A total of 1263 blood disc samples were submitted to the laboratory over a six-month period and an extensive survey of HC antibody titers in the population of wild swine was conducted. Five case reports of herds in which HC infection was suspected were presented where blood disc specimens were utilized in confirming or rejecting a diagnosis of HC.

Introduction
A fluorescent antibody, serum neutralization (FASN) test for the detection of antibodies against HC virus has been found of value in confirming hog cholera (HC) infection in swine. However, in order to obtain serum for this test it was necessary to collect a blood sample from the anterior vena cava. This procedure was not without peril for the pig and was often difficult and hazardous for the veterinarian when a large pig was bled. The swine farmer was concerned when the subject was a pregnant sow or valuable boar.

For these reasons attention was directed to the possibility of utilizing a smaller blood sample that might be collected from an ear vein after superficial puncture with a hoe-bladed scalpel or sharp hypodermic needle. Previous experience by the senior author testing for leptospiral antibodies in blood specimens collected from wild animals on small filter paper discs was the source of an idea for collecting a swine blood sample from the ear vein on a larger paper disc. In addition to the ease of collection, there were some other advantages such as convenient storage since preservation or refrigeration was not required after the blood had dried on the paper disc. Shipment to the laboratory could be accomplished by simply mailing.
The leptospiral antibodies were quite stable during storage on the filter paper disc as long as the discs were kept dry and free of mold growth. Antibody titers were unchanged when serum were eluted from discs stored for a year in a dessicator jar.

This report describes the successful application of the FASN test for the detection of antibodies against HC virus collected on filter paper discs. Preliminary experiments were performed to establish the feasibility of the method and a field study was conducted to standardize the technique. Hog cholera infection as determined by the percent of pigs with blood disc antibody titers was studied in a large group of wild swine. Several case histories were compiled in which the paper disc titers were of value in confirming or rejecting a diagnosis of HC.

**Literature Review**

In 1948 Stapp and Bercks described a method for drying rabbit serum containing antibodies against potato viruses on paper. The dried sera were reconstituted from the paper after storage periods of one and one half years without loss of antibody potency. Adams and Hansen adsorbed vesicular stomatitis antisera on paper discs of commercial blotting paper finding that both positive and negative sera could be eluted from the discs and employed in neutralization tests with chicken embryos. It was found that heat labile nonspecific virus neutralizing substances were destroyed or irreversibly bound on the cellulose fibers. The paper adsorption method was considered suitable for serologic surveys. Filter paper discs were employed by Karstad et al for sampling wild birds for antibodies against eastern equine encephalomyelitis (EEE) virus. The antibody titers from sera adsorbed and eluted from the discs were lower than the untreated sera. However, this reduction did not seriously impair the validity of the test and the method was of value for use in epizootologic studies. Trainer et al collected field samples of whole blood from pheasants on filter paper discs and utilized 100 pheasants in an experiment to simulate environmental conditions likely to occur during specimen collection in the field. There were no significant differences between the antibody titers obtained from the sera and the paper discs except when the bird carcasses were frozen and false positive titers were found. It was shown that the use of the blood disc samples for serological surveillance of EEE was a reliable laboratory procedure.

**Materials and Methods**

Fluorescent Antibody Serum Neutralization Test. - The HC neutralization technique, including cell cultures, indicator virus, serum dilution method, fluorescent antibody conjugate and microscope was described in detail in an earlier publication. Essentially, the FASN test was a fluorescent antibody plaque reduction technique employing a test dose of 1000 virus plaque forming units per 0.1 ml mixed with 0.1 ml of the diluted serum or eluate from the filter paper disc. The eluate-virus suspension mixtures were incubated at 37C for one hour and inoculated on coverslips in Leighton tubes. The coverslip cultures were incubated 18 to 24 hours at 37C and checked for HC virus by the fluorescent antibody technique. The endpoint titer was considered the dilution of the eluate that caused...
at least a 90 percent reduction in plaque count of the virus. A fourfold dilution scheme was employed and antibody titers were reported as logarithms to the base 10 of the dilution, e.g.: 1-4, 0.6; 1-16, 1.2; 1-64, 1.8; 1-256, 2.4; 1-1024, 3.0; and 1-4096, 3.6.

Filter Paper Discs. — The filter paper discs* were paper circles 30 mm in diameter and approximately 1.5 mm thick, capable of adsorbing approximately 1.1 ml of serum or whole blood. The discs were moistened with blood and air dried. In the field it was found helpful to insert a large pin through the middle of the disc prior to wetting it with blood and then mount the disc on a clean piece of cardboard while it dried. The discs were identified by pencil markings or placed in separate marked envelopes prior to mailing to the laboratory.

The blood proteins were eluted by folding the disc and placing it in 4.4 ml of Earle's cell culture medium. The disc was broken up with the tip of a pipette and soaked for 2-4 hours at 37°C or overnight at 4°C. After compressing the paper disc and removing it, the eluate was centrifuged at 2900 rpm on a No. 253 head in a PR-2 (refrigerated) international Centrifuge.** The effective dilution of the serum proteins in the eluate was considered to be 1-8 and a serial fourfold dilution scheme was employed. The final dilutions of the eluate after mixing with an equal volume of test virus were 1-16, 1-64, 1-256, 1-1024, and 1-4096. The FASN test was performed on the paper disc eluates in the same manner as with the serums described above. The elution technique described above was the standard method developed after modification in "Experiments and Field Surveys."

Preliminary Experiments

Heparinized whole blood was pipetted onto the discs in various amounts to determine the maximum volume that could be adsorbed. Another consideration was the possible loss of adsorbed blood during handling and drying. It was found that the discs adsorbed 1.1 ml. of whole blood and yet remained dry to the touch of the fingers.

The effect of chemicals in the paper on the HC virus and cell culture was investigated by soaking untreated discs and discs adsorbed with normal serum in the diluting medium. The eluates from both untreated discs and those with normal serum caused a 50 percent reduction in the plaque count of the virus. Since this was considerably less than the 90 percent reduction required to make the reading positive at the lowest dilution of 1-16, the amount of toxic materials in the disc was considered insufficient to impair the use of the discs for the test.

The next step was to determine whether HC antibodies could be dried on the discs and eluted. Three serums with low antibody titers against HC virus and three blood samples from hyperimmunized pigs were dried on paper discs. An approximate dilution scheme was set up and the neutralization test was performed on eluates from these discs. The antibody titers obtained on the eluates were slightly higher than the titers obtained directly from the serums of these pigs. (Table 1) The antibody proteins of the high titered blood specimens were recovered intact from the discs.

* Catalog Number 900. Carl Schleicher and Schuell Company, Keene, New Hampshire, 03102.
** International Equipment Company, Needham Heights, Massachusetts 03194.
Experiments and Field Surveys

On the basis of information from references and the preliminary experiments conducted, a project was developed in cooperation with the second author to evaluate the filter paper disc technique in two phases. The first part involved collecting replicate specimens, serum and blood disc samples, on the same pigs in herds suspected of HC infection and comparing the titers obtained. Specimens from 162 pigs in 29 herds were collected. No antibodies against HC virus were detected in 83 pigs located in 16 of these herds. The serums were negative at the 1-4 dilution and the disc eluates from these same pigs were negative at 1-16. The accuracy of the negative side of the disc technique was considered established by these findings.

The blood discs from the first seven herds with serum antibody titers were found to have slightly higher titers than the serums. (Table 2) The mean antibody titers for each herd were calculated for discs and serums. The mean serum titer for the 44 pigs was 1.38 and the mean disc titer was 1.50. A paired difference analysis was performed on the data and antibody titer differences were statistically significant (P < 0.01).

Although the disc and serum titers were in fair agreement, a modification was made in the dilution scheme to increase the volume of eluting medium. The discs of the remaining six herds with HC antibody titers processed in the survey were eluted according to this modification and a much better correlation was obtained. (Table 3) The mean serum and disc titers for the 35 pigs were 1.48 and 1.49 respectively and statistically, no significant difference in the two procedures was found.

After the paper disc technique was standardized the next phase was to apply it to confirming the presence of HC infection in suspect herds. For a six-month period, from January to June, 1969, 1263 disc samples were submitted to the laboratory. (Table 4) A fair portion, 171, contained HC antibodies and 1092 were negative. Some of the titers were quite high, 2.4 and 3.0; and were indicative of active HC infection or vaccination with a potent live virus vaccine.

During this period many of the discs submitted were collected from wild swine under difficult conditions. The obvious advantages of the blood disc technique were recognized and the discs were used to determine the serologic status of a population of wild pigs suspected of infection with a HC virus strain of low virulence. The wild pigs were trapped and killed over a period of five months and blood disc samples were collected from a certain number each month. The percent of pigs with HC antibody titers by month varied from 9 to 16 percent. (Table 5) However, there appeared to be no trend up or down in the percent positive during this period. Three conclusions were drawn from this data: A low virulent or subclinical HC infection was not spreading through this population, the HC infection had occurred previously and had been self-limiting, or perhaps some of the pigs had been vaccinated with live virus vaccine before release into the range area. It was suggested that a static situation in regard to HC infection existed in this population of wild pigs and a recommendation was made that another serologic sampling be conducted after a six-month interval. In the meantime, specimens from any sick or dead pigs observed were to be cultured for HC virus.

Case Reports

Pregnant Sow Transmission Confirmed with Blood Disc Samples. – On April
25, 1969, an Animal Health Division Veterinarian located a herd of sows and feeder pigs believed to be the source of HC infected swine sold through the local auction market. All of the pigs on the farm were healthy and had never been vaccinated or treated with HC antiserum. Blood samples on filter paper discs were collected from two feeder pigs and HC antibody titers of 1.8 (1-64) were detected. These titers were considered evidence of HC infection in the herd and close surveillance was maintained.

Eventually, abortions and stillbirths were reported by the owner and on June 24, 1969, tissues were collected from baby pigs four to seven weeks of age. The tissues were submitted to the local laboratory and HC virus was isolated by the fluorescent antibody cell culture technique.* Since the sows were never sick, blood disc samples were collected from them when the herd was depopulated on July 3, 1969. Of nine sows sampled, three had HC antibody titers of 3.0 (1-1024), three of 2.4 (1-256), two of 1.8 (1-64) and one was negative. Hog cholera infection of the sows was indicated by these titers and the abortions and stillbirths were attributed to transplacental HC infection of the baby pigs.

Chronic Illness Not Confirmed as Hog Cholera. — Hog cholera was suspected in a herd of about 50 pigs in which a number of deaths had occurred for the past four months. There were usually about five sick pigs in the herd at any given time. At the time of the visit, rectal temperatures of some of the pigs were slightly above normal, but all of the pigs were eating well.

Blood disc samples were collected from 20 pigs and no HC antibodies were detected in the eluates from the discs. On the basis of this finding, HC virus was rejected as the etiologic agent causing illness and death in this herd.

Acute Illness not Confirmed as Hog Cholera. — On November 14, 1968, sickness in a herd of 657 unvaccinated pigs was reported by the owner. Although there were 120 pigs sick and five had died, no treatment was given. On November 17, 1968, the Animal Health Division Veterinarian was called to the premise because HC was suspected. The sick pigs were coughing, not eating, and had increased rectal temperatures up to 107°F. Some pigs were killed for necropsy and the lesions found were pneumonia, congestion of lymph nodes, petechial hemorrhages on serious membranes and enteritis.

Two live pigs were sent to the local diagnostic laboratory** for examination. Pneumonia and intestinal parasites were found and TWBC counts of 15,200 and 14,350 were recorded. Hog cholera virus was not isolated from the spleens and tonsils by the fluorescent antibody cell culture technique, but histologic lesions consistent with virus pneumonia were reported.

On December 3, 1968, blood disc samples were obtained from five recovered pigs on the farm and no antibodies against HC virus were detected. The diagnosis of HC was rejected and the illness in the herd was attributed to viral or bacterial pneumonia.

Detection of Hog Cholera Exposure Prior to Movement. — After depopulation of a herd infected with HC, the owner reported that three gilts probably exposed to

* C. E. Kord Animal Disease Laboratories, Box 9039, Melrose Station, Nashville, Tennessee, 37204
** Iowa State University Diagnostic Laboratory, Iowa State University, Ames, Iowa, 50010.
the disease had been sold previously. The gilts were traced and located in the new herd in a pen with another gilt. None of the four pigs had clinical signs of HC so blood disc samples were collected to check for HC antibodies. Two of the three gilts moved from the HC infected herd had antibody titers of 2.4 (1-256). The other exposed gilt had a titer of 1.2 (1-16) while the home-raised gilt was negative.

The high titers detected in two of the three gilts were evidence that the pigs had been infected with HC. However, the infecting strain of HC virus was probably of low virulence since no illness was observed and transmission to the home-raised gilt did not occur.

Area Exposure to Hog Cholera not Confirmed. — Hog cholera was suspected on a farm where three sows had farrowed dead and unthrifty pigs during January and February, 1969. The disease had been diagnosed on two of the three adjacent farms on January 13 and February 20, 1969. Tissue specimens collected from these baby pigs were negative for HC virus. In an effort to resolve the status of the herd before releasing the quarantine, blood disc samples were collected from six of the affected sows, none of which had been vaccinated.

Neutralization tests on the eluates from these discs were negative. The absence of detectable HC antibodies at the 1-16 dilution was considered valid evidence that the aborations and stillbirths were due to some other agent.

Discussion

The filter paper disc technique for detecting antibodies against HC virus was found to be a handy means of confirming or rejecting a diagnosis of HC infection particularly under adverse field conditions. The method was as sensitive and accurate as the neutralization test applied directly to the serum samples. The technique was standardized on specimens submitted from the field.

If applied effectively this procedure will aid the eradication of HC since detecting HC antibodies in a pig is like finding the footprints of the virus. The pig has either been infected with HC virus and produced its own antibodies or has received HC antibodies from its dam or the syringe of the local veterinarian. The most effective way to apply the method is to collect paired disc samples at least 21 days apart. The first set of discs could be collected during the initial investigation and the second set just before the quarantine is released or after convalescence of some of the sick swine. At least 10 to 20 disc samples should be collected and the veterinarian should use discretion by sampling according to pen, age group, origin, etc. Of course, when the pigs have been vaccinated, treated with anti-HC serums, or have nursed HC immune sows; it will be necessary to find a rise in titer between the two disc samples to confirm the presence of HC infection. In swine herds where the HC infection has been present for three or four weeks, single disc samples will confirm or reject the presence of HC infection.
THE FILTER PAPER DISC TECHNIQUE

TABLE 1

ANTIBODY TITERS FROM PAPER DISC ELUATES COMPARSED WITH THE TITERS OBTAINED DIRECTLY ON THE SERUMS

<table>
<thead>
<tr>
<th>ADSORBED ON DISC</th>
<th>SERUM TITER LOG 10</th>
<th>PAPER DISC TITER LOG 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>1.2 (1-16)</td>
<td>1.4 (1-24)</td>
</tr>
<tr>
<td>Serum 2</td>
<td>1.8 (1-64)</td>
<td>2.0 (1-100)</td>
</tr>
<tr>
<td>Serum 3</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Blood 370</td>
<td>3.6 (1-4096)</td>
<td>3.6 (1-4096)</td>
</tr>
<tr>
<td>Blood 376</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Blood 377</td>
<td>3.6</td>
<td>3.6</td>
</tr>
</tbody>
</table>

TABLE 2

COMPARISON OF MEAN HC ANTIBODY TITERS OF DUPLICATE SERUM AND PAPER DISC SPECIMENS COLLECTED FROM SWINE IN HC SUSPECT HERDS – EMPLOYING INITIAL DILUTION SCHEME

<table>
<thead>
<tr>
<th>ACCESSION</th>
<th>NUMBER OF SWINE BLED</th>
<th>MEAN SERUM TITER</th>
<th>MEAN DISC TITER</th>
</tr>
</thead>
<tbody>
<tr>
<td>35348</td>
<td>5</td>
<td>1.56</td>
<td>1.94</td>
</tr>
<tr>
<td>35685</td>
<td>1</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>35884</td>
<td>4</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>35885</td>
<td>10</td>
<td>1.20</td>
<td>1.26</td>
</tr>
<tr>
<td>36386</td>
<td>12</td>
<td>0.75</td>
<td>0.95</td>
</tr>
<tr>
<td>36618</td>
<td>10</td>
<td>1.98</td>
<td>2.16</td>
</tr>
<tr>
<td>36810</td>
<td>2</td>
<td>1.80</td>
<td>1.80</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average of 44 Pigs in 7 Herds</td>
<td>1.38</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 3

**COMPARISON OF MEAN HC ANTIBODY TITERS OF DUPLICATE SERUM AND PAPER DISC SPECIMENS COLLECTED FROM SWINE IN HC SUSPECT HERDS – EMPLOYING MODIFIED DILUTION SCHEME (INCREASED VOLUME OF ELUTING FLUID)**

<table>
<thead>
<tr>
<th>ACCESSION</th>
<th>NUMBER OF SWINE BLED</th>
<th>MEAN SERUM TITER</th>
<th>MEAN DISC TITER</th>
</tr>
</thead>
<tbody>
<tr>
<td>36921</td>
<td>4</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>37019</td>
<td>2</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>37088</td>
<td>10</td>
<td>1.32</td>
<td>1.38</td>
</tr>
<tr>
<td>37885</td>
<td>5</td>
<td>2.16</td>
<td>2.40</td>
</tr>
<tr>
<td>38615</td>
<td>5</td>
<td>2.64</td>
<td>2.40</td>
</tr>
<tr>
<td>38827</td>
<td>9</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average of 35 Pigs in 6 Herds 1.48 1.49

### TABLE 4

**SUMMARY OF ROUTINE USE OF FILTER PAPER DISCS BY MONTH JANUARY TO JUNE, 1969**

<table>
<thead>
<tr>
<th>MONTH</th>
<th>TOTAL DISCS RECEIVED</th>
<th>DISCS NEGATIVE FOR HC ANTIBODIES</th>
<th>DISCS POSITIVE FOR HC ANTIBODIES</th>
<th>MAXIMUM TITER OBSERVED</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>58</td>
<td>56</td>
<td>2</td>
<td>1.8 (1-64)</td>
</tr>
<tr>
<td>February</td>
<td>225</td>
<td>190</td>
<td>35</td>
<td>3.0 (1-1024)</td>
</tr>
<tr>
<td>March</td>
<td>170</td>
<td>151</td>
<td>19</td>
<td>2.4 (1-256)</td>
</tr>
<tr>
<td>April</td>
<td>352</td>
<td>300</td>
<td>52</td>
<td>3.0</td>
</tr>
<tr>
<td>May</td>
<td>143</td>
<td>129</td>
<td>14</td>
<td>2.4</td>
</tr>
<tr>
<td>June</td>
<td>315</td>
<td>266</td>
<td>49</td>
<td>3.0</td>
</tr>
<tr>
<td>Total</td>
<td>1263</td>
<td>1092</td>
<td>171</td>
<td></td>
</tr>
</tbody>
</table>
### Table 5

**Paper Disc HC Antibody Titers Obtained from Monthly Samples Collected from a Population of Wild Swine**

<table>
<thead>
<tr>
<th>Month</th>
<th>Pig Titers Positive at 1.2 and Higher</th>
<th>Total Pigs Sampled</th>
<th>Percent with HC Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2</td>
<td>22</td>
<td>9%</td>
</tr>
<tr>
<td>February</td>
<td>34</td>
<td>218</td>
<td>16%</td>
</tr>
<tr>
<td>March</td>
<td>13</td>
<td>132</td>
<td>10%</td>
</tr>
<tr>
<td>April</td>
<td>46</td>
<td>316</td>
<td>15%</td>
</tr>
<tr>
<td>May</td>
<td>14</td>
<td>126</td>
<td>11%</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>814</td>
<td>13.4%</td>
</tr>
</tbody>
</table>
REFERENCES

THE STATUS OF STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

M. J. Tillery, DVM

INTRODUCTION

Fiscal year 1969 was an eventful year in the State-Federal effort to eradicate hog cholera. Marking the seventh year of this activity, most of the states have reached the eradication phases of the program. During the year, State and Federal actions were taken which have eliminated vaccine usage in some states, and which will lead to vaccine no longer being used to control hog cholera in the remaining states in the near future. While there was both program advancement and a sharp decrease in vaccine usage, hog cholera incidence increased in some localities, and this was expected by those administering this effort. However, no widespread increase has occurred, and the program continues to have the desired effect on hog cholera incidence nationally.

The purpose of this report is to review some of the events of these years and thereby to call attention to some of the program’s successes as well as some of the problems which must be solved in order to achieve the ultimate goal of hog cholera eradication.

PROGRAM STATUS

By July 1969, most states had completed the transition from hog cholera control, represented by Phase II, to hog cholera eradication represented by Phases III and IV. Only 6 states, none of which are major swine-producing states remained in Phase II, Twenty-seven states were in Phase III, and 17 states were in Phase IV. Twelve states were hog cholera free. Some changes occurred within the interval between July and this meeting of the U.S. Animal Health Association which appear significant to this effort. West Virginia achieved hog cholera free status; however, Michigan lost free status due to the spread of hog cholera to three herds after being introduced from another state. Therefore, 12 states remain hog cholera free. Three states have advanced from Phase II to Phase III, leaving Maine, New Hampshire, and New York at the Phase II level. Tennessee advanced to Phase IV, being the fourth southeastern state to reach that level.

Several things should be pointed out here. First, a large area of the West has virtually completed this program and continues to operate at Phase IV or hog cholera free. While this area has a relatively small swine population, these states’ continuing ability to maintain these levels of the program is additional evidence that the effort can be successful. Further, maintenance of these levels was achieved in the face of hog cholera having been introduced in several instances. These events, while exciting concern among those involved, were eliminated by application of those measures specified for these program levels, and this large area continues to

be virtually hog cholera free.

The heavier swine-producing areas of the North Central States and the Southeast are in Phase III for the most part, although a few states in these areas have reached Phase IV or even hog cholera free status. Phase III is probably the most important level of this program. For in Phase III, hog cholera is eliminated. While some of these states continue to have relatively high hog cholera incidence, outbreaks in most of them are sporadic and quickly contained and eliminated. In those few states which have experienced heavier incidence, program standards are being utilized to eliminate these outbreaks. In some, the rapidly spreading situation appears to have been contained and eliminated. In others, the buildup appears to have been contained; and the campaign continues to eliminate remaining foci of infection. Evidence mounts to demonstrate that any fear that Phase III of this program cannot eliminate hog cholera from an area is without scientific foundation.

Some states continue to operate at lesser levels than their hog cholera experience warrants. Therefore, those states, as well as others which achieve similar successes in eliminating hog cholera, should take advantage of these situations and forge ahead just as quickly as possible. Else, there is possibility that the effort will become static with resulting loss of interest and enthusiasm for the ultimate objective of eradication of hog cholera.

INCIDENCE

In fiscal year 1969, hog cholera occurred in 427 counties in 34 states and
Puerto Rico. Eleven states have had no hog cholera for at least two years. However, some states which had previously experienced little hog cholera had increased incidence.

In fiscal year 1969, 1055 infected herds were disclosed compared to 849 in 1968. The 1969 incidence represented the third year of such experience since 1966. This situation continues into fiscal year 1970. Several factors appear to be at work here.

One if that reporting of suspicious outbreaks continues to improve. Probably, the expansion of the program to Phases III or IV with the concurrent availability of indemnities has resulted in more reports of suspicious illnesses by swine owners. With reports increasing at a greater rate than confirmations, the earlier reported incidence might be challenged.

However, assuming that the 1966 information is correct, that year would have been the best time yet experienced for the country to have moved to the Phase III level or higher of this program. But, all were not prepared to take such a step in 1966. Earlier experience indicated that remaining at Phase II or lower for prolonged periods leads to increased outbreaks. Therefore, these increases would have been reduced or avoided by earlier program advancement.

Another factor related to these increases is the disclosure of more discreet forms of hog cholera. Mild infections are reported today. In other instances, lengthy incubation periods of 60 days or more are commonly encountered. These added skills in diagnosis have contributed to an apparent increase in outbreaks.

Yet, another factor is an increase in outbreaks in a few states. In 1969, six states, Florida, Georgia, Missouri, North Carolina, South Carolina, and Texas, accounted for 591, or 56 percent of the infected herds. The remaining 44 states and
Puerto Rico had less than one-half the hog cholera, or 464 outbreaks.

However, rationalizing this situation could lead to assumptions which would endanger this program. The fact is that an increase in outbreaks has occurred which could have been prevented had we been better prepared to act in 1966. All were not prepared. However, most of the country is now moving with dispatch against hog cholera. Even so, all must recognize a potential for other increases in hog cholera. All must realize that any state, or any locality containing swine within a state, is vulnerable to a rapid increase in outbreaks. All must be alert to these potentials and use whatever resources necessary to eliminate that hog cholera which occurs so that these potentials are not realized.

With this in mind, the Animal Health Division announced on June 4, 1969, that the Division would consider the hog cholera eradication program an emergency activity. Further, this program would have first priority on the Division’s funds and personnel. Eight conferences were held with State and Federal program officials at the end of fiscal year 1969 to discuss these aspects of the effort. Both field and headquarter personnel in the Division are now operating on an emergency basis.

**EPIDEMIOLOGY**

A basic function of eradication program officials is analysis of the epidemiology of the disease in question. Hog cholera eradication is no exception, and
considerable epidemiological information is now at hand. Certain trends in hog cholera epidemiology can now be identified, and this was not the case in the early years because the necessary data were not available.

A major means of spread of hog cholera continues to involve marketing. Further, the proportion of outbreaks spread in this fashion has increased. Interstate movements accounted for little spread of hog cholera in earlier years. However, hog cholera moving through these channels increased in 1969. Area spread has been a major means of transmission.

All of these methods of spread are susceptible to quarantine activities and the subsequent elimination of infected herds. In earlier years, quarantine was limited to individual premises for the most part. However, statewide quarantines, with accompanying closing of markets to swine and similar measures were used to attack the spread of hog cholera for the first time in 1968, and this procedure was utilized more widely in 1969. These measures, first used in Georgia and subsequently in Arkansas, Florida, the Carolinas, Missouri, and elsewhere were highly effective against hog cholera.

A similar measure used for the first time in this program in July 1969 was Federal quarantine. These restrictions were applied to the Delmarva Peninsula. Again, it appears that these actions assisted in limiting the spread of hog cholera.

In retrospect, it appears that the highly effective maneuver of quarantine, or standstill order, could be used to even better advantage in this program. Generally, it appears that his measure was delayed because of its drastic effect on swine...
marketing. These delays provided ample opportunity for the hog cholera situation to worsen. Therefore, it appears that such area-wide measures as State and Federal quarantine should be applied quicker in order to be of maximum effect.

The same situation holds true with Federal quarantines. With regulations supporting these measures having been amended so that administration of quarantine is more practical, Federal quarantines will likely be utilized more readily in the future. Such quarantines should have a telling effect now that the heavier hog cholera incidence is limited to six or fewer states.

Hog cholera associated with vaccination dropped to a low of four percent in 1969. This is undoubtedly the result of State and Federal restrictions on vaccine use. By July 1, 1969, 39 States had virtually eliminated vaccine usage from their programs. In addition, Federal regulations had been enacted which practically eliminated further interstate shipment of vaccines. Because of similar Federal restrictions on their interstate movement, few, if any, swine should be vaccinated after January 1, 1970.

These actions are the direct result of program experience with hog cholera vaccines. Further, the Federal actions are the result of requests by cooperating State officials, industry leaders, and scientists. This is important. For by making such requests these interests have finally indicated their desire to abandon a costly effort at hog cholera control in favor of hog cholera eradication.

Hog cholera spread by garbage feeding continues to increase. Some actions have been taken to eliminate this practice. Others are planned. In the meanwhile, all pressures available must be brought to bear on the individuals who choose to ignore existing laws and regulations concerning garbage feeding. These individuals not only endanger the hog cholera eradication program, but they invite the introduction of even more costly diseases now foreign to this country. Such irresponsible actions by a few individuals need not be tolerated any longer. Therefore, efforts to eliminate feeding of garbage to swine will likely continue.

An area of considerable satisfaction to those administering this effort is the steady increase in the skill of identifying the methods of spread of hog cholera. This skill is illustrated in the steady decline of no probably source cases. Having reached a low of 6 percent, it is doubtful that the ideal of identifying sources of infection of all outbreaks will be achieved. However, this ideal must be sought, for identifying the spread of the disease is one of the major activities of a program such as hog cholera eradication. Only by such identification can program administrators apply their resources in a logical manner. Too, the identification of means of spread in the bulk of the outbreaks further invalidates the possibility of unknown reservoirs of hog cholera virus.

**OUTLOOK**

Hog cholera eradication continues to be feasible. Also, those principles of a State-Federal effort toward this objective, first laid down in 1962, continue to be sound, realistic, and practical. While some individuals have failed these principles in the past, never have the principles failed when applied. Further, it appears that incidence of hog cholera has been reduced so that sporadic outbreaks occur although incidence can be rather heavy as illustrated by six states having over
one-half the hog cholera in 1969. Therefore, it appears that such storms of outbreaks may be expected in any locality, but no reason has yet been exposed which would prevent the elimination of these incidents by vigorous application of the principles outlines by this Association. It remains, then, to apply these actions with whatever enthusiasm necessary to eliminate the economic waste attributable to hog cholera as quickly and thereby as economically as possible. This urgency for program completion is directly in line with recommendations made by this Association on several occasions.
REPORT OF THE COMMITTEE ON
THE NATIONWIDE ERADICATION OF HOG CHOLERA

United States Animal Health Association
June 13, 1969

Chairman: P. B. Doby, Springfield, Illinois

Due to the changes which will result in the National Hog Cholera Eradication Program with the implementation of Federal regulations prohibiting or restricting interstate movement of hog cholera vaccines after July 1, 1969, the Committee felt the necessity of holding a midyear meeting. This meeting was held in Columbus, Ohio, June 13, 1969. Deliberations at this meeting dealt principally with improved control of swine movements.

Committee recommendations:
1. The chair shall appoint a sub-committee to contact and work with the USAHA Committee on Livestock, Markets and Transportation to attempt to tie hog cholera market standards in with over-all market standards.
2. The Committee accepted the proposal on Phase II market standards and recognized it as an ideal means of administering market regulations. Further, the Committee recognized that all States may not be able to immediately staff such an operation. In those instances, those States would suspend all marketing operations when veterinary investigation reveals that hog cholera is being spread through markets in epidemic proportions. Such suspension would be maintained until the epidemic is brought under control. (See addendum No. I)
3. The Committee accepted the proposal on Phase III market standards, except that the procedures under B-1 would be put into effect only in those areas under area-wide quarantine. (See addendum No. II)
4. The Committee recommended increasing maximum Federal indemnity payments for grade animals from $40 to $50 per head.
5. The Committee recommended that salvage in Phase III be discontinued at the time of stopping vaccination (January 1, 1970) and when the Federal government will provide indemnity funds for pigs in interstate commerce and for States which have exhausted their own funds for indemnity.
6. The Committee endorsed the series of eight regional meetings of regulatory officials on the change of emphasis on the hog cholera eradication program and recommended inviting representatives of the State veterinary medical associations in those States involved in each meeting.
7. The Committee recognized the need for exchange of laboratory equipment...
and personnel between States in emergency diagnostic situations.

(8) The Committee encouraged the stockpiling of anti-hog cholera serum and reaffirmed that live hog cholera vaccines not be used as a part of the eradication program. (Vote 5-4 in favor)

(9) The Committee recommended adoption of the Guidelines for Voluntary Supervised Swine Herds as a part of the eradication program. Operation of this will be reviewed this fall at the regular meeting of the Committee. (See addendum No. III)

ADDENDUM NO. I

Phase II — Reduction of Incidence

A. Quarantines

1. ****
2. ****
3. ****

B. Livestock markets, buying stations, and concentration points handling all classes of swine:

1. All swine, including slaughter swine, to be inspected by an accredited veterinarian prior to leaving market.
2. Swine moving interstate from markets to be in compliance with Part 76, Title 9, CFR, including health certification by the accredited veterinarian authorized by the State to furnish such services.
3. Slaughter swine leaving premises to be consigned only for immediate slaughter to a recognized slaughtering establishment approved for this purpose in accordance with Federal and State regulations.
4. Markets to maintain well-constructed pens and swine-handling facilities that are clean and in good repair.
5. Markets to provide pens surfaced with concrete for holding and handling feeder pigs and breeding swine.
6. Markets to provide satisfactory, well-lighted facilities for inspection and proper restraint.
7. Holding and handling pens, alleys, and other facilities used in selling swine shall, after use by each lot of swine, be cleaned and disinfected under procedures specified by State and Federal agencies to guard against spread of disease.
8. Arrange for adequate facilities and service at a nominal cost for cleaning and disinfecting cars, trucks, and other vehicles with permitted disinfectant when this is required for movements as required by regulations, or deemed necessary by regulatory officials.
9. Maintain records of origin and destination for all swine entering market, and grant Federal and State inspectors access to such records. Identification as to farm of origin shall be maintained for all feeder pigs and breeding stock as well as all slaughter swine which may be diverted for purposes other than slaughter. Records shall be maintained for 1 year.
10. Provide a schedule of sale days.
C. Livestock markets, buying stations, and concentration points handling slaughter swine only:
   1. Swine moving interstate to be in compliance with Part 76, Title 9, CFR, and applicable State regulations.
   2. Accept only swine for slaughter and to permit no swine to leave market except in compliance with No. B,3, above.
   3. Markets to maintain well-constructed pens and swine-handling facilities that are clean and in good repair.
   4. Maintain records of origin and destination for all swine entering market and grant Federal and State inspectors access to such records. Records shall be maintained for 1 year.
   5. Isolate all swine suspected of being affected with or exposed to hog cholera, promptly notify the State or Federal agency, and hold such swine in isolation pending instructions on disposition.
   6. Holding and handling pens, alleys, and other facilities used in selling swine shall, after use by each lot of swine, be cleaned and disinfected under procedures specified by State and Federal agencies to guard against spread of disease.

D. Regulatory activities at markets, buying stations, and swine concentration points dealing in all classes of swine:
   1. Provisions to be made for a State or Federal livestock inspector to enforce State and Federal requirements at all such markets, and be in attendance throughout all transactions involving swine entering and leaving markets.
      a. Livestock inspector to ascertain that only thrifty, qualified swine enter market.
      b. Swine not qualified to enter market are to be directed to slaughter facilities or returned to owner’s premises under quarantine and State/Federal escort arranged by inspector.
      c. Livestock inspector to ascertain compliance with State and Federal requirements for all swine leaving market.
      d. Livestock inspector to immediately report to State and Federal regulatory officials any deviation from market standards (facilities or operations).
      e. State and Federal officials to promptly correct any deviations noted.

E. Regulatory activities at markets, buying stations, and swine concentration points dealing in slaughter swine only:
   1. Provisions to be made for a State or Federal livestock inspector to examine records and facilities of each such market at least once a month for compliance.
   2. Any deviation from market standards are to be reported to State and Federal regulatory officials immediately.
   3. State and Federal officials to promptly correct any deviations noted.
III. Phase III — Elimination of Outbreaks

A. A statement will be submitted, through the Assistant Director covering the following points in detail:

1. All steps in Phase I and Phase II have been or are being carried out effectively.

2. 

3. 

4. 

5. etc.

B. Livestock markets, buying stations, and swine concentration points handling all classes of swine:

1. Permit no swine to enter market unless accompanied with an official inspection certificate. Such inspection shall be made on premises of origin within 10 days of sale by a person designated by the State and Federal agencies. The inspector shall designate the swine to be sold and identify them in a distinctive manner. Any swine in the vehicle at the time of consignment to the market which are not so identified, shall cause the inspection to be void and all such swine shall be ineligible for marketing that date. The entire load of such swine shall be ordered slaughtered or returned to origin. The State and Federal agencies shall take appropriate action to insure that such swine reach the ordered destination. (To be required only in those areas under area-wide quarantine.)

2. Permit no “cull” pigs to enter market unless provisions are made to pen such pigs separate and apart from all other swine so contact with healthy swine does not occur. Facilities used by these swine will not be used by other swine until cleaning and disinfecting has been accomplished. Further, cull swine to be permanently identified by a round hole punched in the right ear, quarantined to the purchaser, and released from said quarantine by consignment to slaughter only. (A “cull” pig is defined as one which sells for appreciably less than the normal price for pigs of that weight.)

3. Permit no garbage fed swine to enter market unless provisions are made to handle and pen such swine separate and apart from all other swine to avoid contact with other marketable swine. Facilities used by these swine will not be used by other swine until cleaning and disinfecting has been accomplished. Further, that such swine be identified by a hole (approximately 3/8” to ½” in diameter) punched in the right ear and consigned for slaughter only. (Swine which have been fed raw garbage can move interstate only for special processing in accordance with 76.13 and 76.17, CFR.) Inspector will supervise and ascertain that garbage fed and/or cull swine comply with the market standards as outlined above.

4. Permit no swine to be moved into or from the market unless a State or Federal inspector releases such swine.

5. Require all buyers of swine to determine the purpose of their movement. If for slaughter and there is any reason to believe the swine might be diverted (underweight swine, thin sows, etc.), the inspector should require that such swine be identified by a hole
(approximately 3/8” to ½” in diameter) being punched in the right ear of such swine prior to release. If for feeding, breeding, or exhibition require individual identification by ear tag and a yellow paint mark. If such pigs have entered the market already so identified, an additional yellow paint mark shall be applied. Any swine which have a round hole in their right ear shall be ineligible for movement except to slaughter. Further, any swine with which these swine mingle shall cause the entire lot to be ineligible for movement except to slaughter.

**ADDENDUM NO. III**

**Guidelines for Voluntary Supervised Swine Herds**

The guidelines herein described are designed to limit the spread of hog cholera. An owner adhering to these measures could afford protection to his herd and, should disease gain entrance, depopulation procedures might be limited to only those swine considered to be at risk. Strict adherence to each item is essential so that a maximum number of swine are afforded protection against exposure. Deviations would invalidate the status of the various segments and could necessitate total herd depopulation on confirmation of hog cholera in any part of the herd.

**Management:**

1. **Segmented Unit** — A herd is divided into isolated units in order to confine disease to as small a group as possible should disease gain entrance into a herd.
2. **Isolation** — No units shall join each other. In each instance a barrier such as a canal, public road, or double fenced vacant area shall be provided between units.
3. **Caretakers** — Each unit has assigned caretakers that service only one unit and at no time enter or service other units, or; caretakers change outer clothing and footwear prior to leaving each unit and in addition disinfect footwear with approved disinfectant prior to entering each unit.
4. **Equipment** — Assigned to one unit and at no time be used in servicing other units. (Should large equipment, such as trucks, be used to service multiple units, the undercarriage and wheels are to be disinfected prior to entering each unit.)
5. **Quarantine** — Swine moving out of a unit will be quarantined separate and apart from all other swine for a period of 10 days prior to moving into another unit.

All swine which are imported into the herd from outside sources will be quarantined on arrival and penned separate and apart in a special quarantine facility used only for such purposes. The swine imports will be released from quarantine only if found healthy on veterinary inspection following a minimum 30-day quarantine period.

6. **Veterinary services of an accredited veterinarian are to be utilized whenever such services are deemed necessary by the owner or the regulatory veterinarian supervising the herd.**
7. **Regulatory Supervision** — A regulatory State-Federal Veterinarian will inspect the entire herd at least once every six months for health and compliance and other times as appropriate. Inspections are to be concentrated during the spring and during the fall of each year at a time most likely coinciding with peak swine population on the premises and at
other times as appropriate.

8. Records — Complete records are to be available to the regulatory supervising veterinarian and are to include all transactions of movements into and out of the herd as well as origin and destination of swine so moved.

9. Identification — All swine are to be individually identified and identification maintained at all times.

10. General — Waste (garbage) consisting in whole or in part of animal waste resulting from handling, preparing, cooking, and consuming of food including the offal from animal carcasses or parts thereof, including waste from ordinary household operations (table scraps), will not be fed to swine under any circumstances.

Swine which die or are moribund will be removed from the unit immediately to preclude any possibility of cannibalism.

Sanitation:

Every attempt will be made to maintain strict sanitation. Feeding equipment, waterers, and similar equipment will be cleaned and disinfected each time a unit is vacated and prior to introducing a new group of swine. Fly and rodent control will be practiced to keep such populations to the very minimum. Dead animals will be disposed of by burning, burial, or rendering.

Disease Reporting:

The herd owner or his agent will inspect the entire herd at least once a day in an effort to locate any sick swine. Any and all swine showing clinical signs of illness are to be immediately isolated from healthy swine.

In the event more than one animal in any unit exhibits signs of illness, the owner or his agent will notify the Federal-State supervising veterinarian and, in addition, will obtain the services of an accredited veterinarian for an immediate diagnosis. The accredited veterinarian will obtain specimens from any pigs so examined, except those in which clinical findings indicate that the condition cannot possibly be hog cholera. Specimens so collected will be sent to a State or Federal (NADL) diagnostic laboratory.

A record will be kept by the owner of all sickness noted which will be available for State-Federal supervising veterinarians and will include:

1. Number of animals sick in each unit;
2. Identity of sick animals, including unit where found;
3. Date and hour sickness was noted;
4. Clinical signs noted;
5. If and when accredited veterinarian was notified;
6. Veterinarian’s diagnosis, if any;
7. Disposition of sick animals;
8. Disposition of dead animals.

Disease Control:

Should an infectious or contagious disease be diagnosed in any unit, the following procedures will be carried out:

1. All movements of swine will immediately cease and further movements of any swine will not be made unless State-Federal veterinarian authorizes such movement.
2. Immediate notification of State-Federal supervising veterinarian.
FIELD EVALUATION OF THE CHEMICAL TEST FOR PROPER HEAT TREATMENT OF GARBAGE

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SUMMARY

A field test for monitoring proper cooking of garbage has been subjected to successful field trial. The test is expected to receive wide application, particularly in the Cooperative State-Federal Hog Cholera Eradication Program. Field work has indicated that the test can be reliably performed by field inspectors. The test is reproducible in the field and can be accurately applied to distinguish between properly cooked, partially cooked and uncooked garbage. Garbage may be sampled, from the cooker, many days after cooking with great assurance that the test will still give a valid index of heat treatment.

INTRODUCTION

Recently, a simple and rapid field test was developed which may be used to determine whether garbage has been cooked sufficiently for safe feeding to hogs.1 It was required to check the reliability of this test under actual field conditions. This paper reports and evaluates results of the use of this test in the State of Maryland.

Incompletely cooked or raw garbage is a known transmitter of contagious diseases among livestock. In the United States, such diseases include hog cholera, vesicular exanthema, swine erysipelas, trichinosis and tuberculosis.2 For example, during 1968 approximately 12% of hog cholera outbreaks in the U.S. were attributed to feeding raw garbage.3 The field test was devised primarily to assist the State-Federal Hog Cholera Eradication Program.

Garbage cookers are periodically checked to see that proper temperature has been attained. For this purpose, 4 foot probes fitted with a water temperature gauge are presently being used. Therefore, it is necessary for an inspector to be at the cook-site during actual cooking operations. This requires tight beforehand scheduling and adds to the burden of the very busy livestock inspector.

State and Federal regulations stipulate the garbage must be heated throughout to boiling (212 degrees F at sea level) for a minimum of 30 minutes. The field test is designed to monitor this heating requirement. It will give a valid index of 30 minute heat treatment of 200 degrees F, even under minimal test conditions.1 Thus, the test encompasses the inactivation temperatures for the various disease agents listed in Table 1. These are some of the more dangerous disease agents that might be found in uncooked garbage. General heating requirements for meats inspected and passed for cooking are 170 degrees F for 30 minutes.4

The field test has the great advantage that is not necessary for inspectors to be present during actual garbage cooking. The principle of the field test is based on monitoring the effect of cooking upon the survival of the common enzyme, acid
phosphomonoesterase. This enzyme is found in varying degrees in almost all raw animal and plant tissues. It is heat-labile, being progressively inactivated with progressive heating above 110 degrees F, and is completely inactivated at temperatures of 200 degrees F and above.

The technique of the test is to incubate garbage juice with the substrate phenolphthalein monophosphate. The enzyme, if it is present due to insufficient garbage cooking, acts upon the substrate to split-off the single phosphate group, leaving behind the phenolphthalein molecule. The latter produces a red color upon addition of an alkaline buffer ("Solution C"). The presence of the color indicates insufficient cooking and its intensity gives a relative assessment of the degree of under-cook.

MATERIALS AND METHODS

Reagents. (a) "Solution A" — Distilled water. (b) "Solution B" (PMP) — Add 2.00 g phenolphthalein monophosphate (General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N.J.) to 1.00 L citrate buffer, pH 4.8 ± 0.1. The latter buffer is prepared as follows: Dissolve 217.0 g trisodium citrate .2H2O plus 60.6 g citric acid .H2O in about 950 ml distilled water; sterilize citrate buffer by gentle boiling for 15 minutes. Cool and adjust to 1.00 L. Mix well to dissolve PMP; do not use heat. The solution is stable indefinitely when frozen. (c) "Solution C" (Color Producer) — Glycine plus sodium hydroxide buffer, about pH 11.8, prepared as follows: Add 670.0 g glycine (aminoacetic acid, 99.5% minimum purity) to 360.0 g sodium hydroxide flakes and then add about 300 ml distilled water; stir, cool, and adjust to 1.00 L.

Apparatus. Materials for the field test kit are illustrated and described in Fig. 1. The kit is usually equipped with sufficient supplies for about 14 tests.

Method for field test. (Taken from field instructions.)

Caution: Freeze Solution B when not in use. Allow to defrost at room temperature and shake before use. Take along only the amount needed per day and keep cold (in styrofoam box).

Preparation for Test: At start of each day, check Solution B as follows: To test tube add ½ ml Solution B, 1 ml Solution A and 1 ml Solution C. Mix and check color of tube against white background. If tube shows pink, then Solution B is no longer good; replace and freeze or keep cold.

Test procedure: Plug in 12-volt incubator block about 30 minutes prior to test. Regulate the block to obtain any temperature from 90 to 110 degrees F.

Step No. 1, Sampling. Load double layer of cheesecloth with about 1 cupful of well drained garbage. Take samples from beneath surface of garbage and from a number of places in large cooker. Remove any paper, bones, metal, etc. Squeeze out at least 3 ml of clear garbage juice into a clean disposable vessel.

Step No. 2 Reaction. Use 2 test tubes and identify as tube No. 1 for background (control) and No. 2 for garbage (unknown). To test tube No. 1, add ½ ml Solution A. To test tube No. 2, add ½ ml Solution B. To each tube, add 1 ml garbage extract. Mix and incubate both tubes for 15 minutes or more, but not longer than 50 minutes.

Step No. 3, Color Development. At end of incubation, add 1 ml Solution C to each tube.
Step No. 4, Interpretation of Results. Remove tubes and read test against a
white background. Look for red color in test tube No. 2 at the interface about the
middle of the tube where the garbage and Solution C meet. Using a clean glove,
place finger over each tube and invert a few times to mix. Look again for red color
in test tube No. 2.

If tube No. 2 shows a red color (even a trace, or light pink) over that color
existing in tube No. 1, either before or after mixing, then this indicates insufficient
cooking of garbage; therefore, a positive reaction.

Don't mistake a darker color in tube No. 2 over that of tube No. 1 for a
positive reaction; the color must have a red characteristic to be positive.

Occasionally red will be superimposed on a very dark brown. Red is then very
difficult to detect. In such a case, add Solution A to top of tube without mixing;
look for red color at interface.

If not sure of color, repeat the test and increase reaction time to 30 minutes or
more, but not longer than 50 minutes. Incubations longer than 50 minutes may
cause breakdown of Solution B and result in false positives.

If tube No. 2 shows no red or reddish color compared to tube No. 1, it
indicates a negative reaction, i.e., proper cooking.

Additional precautions. (1) Cap reagent bottles immediately after removing
reagents for test. (2) Do not allow Solution C to freeze. If it freezes in the field,
thaw under hot water and shake before using.

Field test trials. Field trials were conducted over a 7 month period during the
late summer through the winter 1968-1969 by two federal livestock inspectors in
the State of Maryland. Laboratory work, including back-up testing, preparation of
reagents and equipment, etc., was performed at Technical Services, Animal Health
Div., ARS, Agricultural Research Center, Beltsville, Maryland.

Field tests were conducted, in most instances, in accordance with the foregoing
instructions for the field test except that all liquid volumes were doubled and larger
equipment to accommodate these volumes was used.

Purposes of the field trials. One purpose of the field trials was to test the
reliability and stability of reagents and the ease of manipulation of the test
equipment. It was found that the test equipment lent itself to easy test
performance. The reagents also were stable within limits and with the precautions
noted in the above instructions. Color Producer (“Solution C”) was stable
indefinitely and upon inadvertent freezing could be readily reconstituted by
warming in water and shaking. However, PMP (“Solution B”) requires special
precautions to minimize breakdown: The solution must be protected from light by
storage in an amber bottle. Refrigeration (ca. 42 degrees F) will result in a storage
life of about 3 to 4 weeks, whereas freezing (ca. -2 degrees F) effectively controls
breakdown.

The main purpose of the field trials was to test the reproducibility and
accuracy of the test in the field and in the hands of chemically untrained workers.
Two field men were taught the test at the laboratory. In every case, the field
workers collected an exact duplicate of the garbage juice for cross-check
verification of field results by the laboratory. The juice was held tightly capped and
refrigerated until submission to the laboratory. It had previously been shown that
garbage juice samples could be kept refrigerated without any change in reaction to
the field test. During these trials, samples were kept sealed and cold as long as a month before submission for laboratory test, with complete agreement between field and laboratory results.

Another important purpose of the field trials was to look for and trace any possible sources of interference with the field test. "Recontamination", in particular, was given attention. By "recontamination" is meant the re-introduction of active acid phosphomonoesterase from either external or internal sources. The establishment and determination of the time required for "recontamination", then, was a measure of the temporal terminus of validity of the field test. Results of various tests for likely interferences and for "recontamination" are given below.

Ancillary to the above purpose was an attempt to determine how widely the field test could be applied. For example, would the test still give valid results on cooked garbage dumped in the hog feed lot, the hog feeder, pen and other sites around the cooker?

RESULTS AND DISCUSSION

The chemical nature of the field test is such that safeguards against interference from certain garbage contents are built-in. For example, many of the field garbage samples contained red beets which imparted a deep red coloration to the garbage juice. This did not interfere because beet juice turns amber upon addition of "Solution C". Further, lye is often added to a garbage vat in order to "sweeten" the contents. Again, there is no interference with the test because "Solution B" contains sufficient buffer action to overcome the effects of added lye at least up to pH 9 to 10.

Table 2 presents data from some garbage samples subjected to the field test before and after thorough cooking. Note that in all cases the test accurately distinguishes between cooked and partially cooked or raw garbage. Sample No. 33 apparently indicates that "recontamination" has occurred 2 days after cooking, however, since no temperature readings were taken during cooking operations, no conclusions regarding "recontamination" could be made.

The data of Table 3 also give evidence that thorough garbage cooking can be readily determined and that various stages of cooking can be distinguished. Sample No. 50 appears to indicate "recontamination" 1 day after cooking but, once again, the highest cook temperature was not known and, therefore, this datum offers no firm conclusions.

All samples for Table 3 were taken on a random basis, each at a different hog farm except for No's 10 and 35 and No's 18 and 28; these were taken at the same two farms but at different times. All garbage was claimed to have been properly cooked, but out of a total of 19 samples, 11, or 58%, gave positive reactions to the field test.

Samples are listed in Table 4 according to sampling site for ease in reading. There appears to be no relationship between the site of sampling in the cooker and the result of the field test; this is also apparently true for the cooking method (direct fire or steam) and for the source of the garbage. Although "recontamination" is expected to be most likely to occur at the top of a standing cooker, and most samples taken from this site were positive, yet one sample, No. 32, showed a negative reaction to the field test even 5 days after cooking. If the top of the
cooker tests negative, then samples taken from the bottom of a cooker, particularly a direct fire cooker, would also be expected to test negative. This may or may not be true of steam cookers depending on the position of the steam outlets and the geometry of the cooker.

Tables 2, 3 and 4 show complete agreement between field and laboratory results. The qualitative assessments of the degree of positiveness, indicated by + signs, are a highly subjective judgement, yet they also show good concurrence. The intensity of the red color obtained will increase with increased incubation time, i.e., increased exposure of "Solution B" to garbage acid phosphomonoesterase. The positive values given in the tables are for an approximate incubation time of from 15 to 30 minutes. It should be stressed that the field test is designed to test for completeness of cooking (i.e., no red color - neg.); any red (pos.) color indicates a potentially dangerous situation, and is sufficient evidence that garbage should be recooked.

In order to check when "recontamination" would be most likely to occur, two experiments were formulated. One was a short term, 24 hour, experiment designed to determine if "recontamination" would occur within this time. Results of this experiment are summarized in Table 5. Temperature readings and large samples were taken during cooking and the field worker satisfied himself that the field test yielded an initial negative result. The sample was then stored in a large clean can or pail at ambient temperature and the field test was conducted again at 24 hrs. In some cases, duplicate samples were taken and one of the samples was tightly covered. The latter was done in order to assess the possibility that anaerobic bacteria might effect "recontamination". It may be seen from Table 5 that in no case could 24 hr. "recontamination" be elicited after thorough cooking.

The longer term experiment was designed to determine when, on the average, "recontamination" could be expected to occur. This experiment is summarized in Table 6. The design of the experiment was the same as that for Table 5 except that the fully cooked garbage was sampled and tested at the indicated intervals to see when the initial negative reaction became positive, i.e., showed "recontamination". Cold weather was expected to retard "recontamination", and hot weather to hasten it. Even so, garbage could be kept for up to 4 days without indicating "recontamination" under all weather and temperatures, at least with the simulated field conditions, i.e., on the livestock inspector's premises. Whether the same results would apply to garbage sites around hog pens was a moot question and was further investigated below. The results of Table 6 indicate that the field test may be applied to a properly cooked garbage kept in the cooker for 4 days after heating with some assurance that the test would give results comparable to those at zero time, i.e., when cooking was considered completed.

During the course of random garbage sampling, five samples were obtained from various sites in different hog pens. Field test results on these samples are given in Table 7. The garbage was claimed to have been properly cooked prior to dumping and since all results indicated high positive reaction, possible sources of contamination were investigated. First, pig feces and a number of mixtures of feces with a known properly heated garbage were subjected to field test; results are recorded in Table 8. Note that hog feces is high in acid phosphomonoesterase and gives a very positive test even when diluted with cooked garbage. Plain garden soil
does not affect the test.

Table 9 shows the results of test when fully cooked garbage was added to the hog feeding trough. Note that garbage dumped into a feeder containing some old garbage (Sample No. 56) shows acid phosphomonoesterase contamination in one hour, and probably immediately. Sample No. 59 shows that cooked garbage dumped into an ostensibly clean feeding trough rapidly indicates "recontamination" when exposed to hogs. Thus, fully cooked garbage which comes in contact with hogs, their droppings or old garbage may be expected to become recontaminated and a valid field test can not be obtained by sampling such swill.

Well over 100 samples were field tested and in each case field results were confirmed by the laboratory. However, at the beginnings of the field trials a few misinterpretations of the results of the field test occurred. Precautions against these have been written into the instructions for the field test. To recapitulate, a darker color in tube No. 2 (unknown sample) over that in tube No. 1 (background control) is not a positive test reaction; the color must have a red characteristic to be positive; in case of doubt about color, the test should be repeated and the reaction time increased to 30 minutes or more, but not exceeding 50 minutes.

CONCLUSIONS

Evaluation of the foregoing data results in the following conclusions:

1. The test can be reliably performed by field livestock inspectors. Evidence for this conclusion is given by the complete agreement between field laboratory results in over 100 field tests. However, it is advisable to institute a training program since there is a possibility of misinterpretation of results.

2. The test accurately distinguishes between cooked, partially cooked or raw garbage. All the data confirm this conclusion, particularly the data of Tables 2 and 3. The test has also been shown to be completely reproducible in the field even after prolonged cold storage of the garbage juice sample.

3. Apparently the field test is not influenced by up to 4 days standing in the cooker open to any kind of weather. Thus, the livestock inspector may sample from the cooker within this time and anticipate valid field test results, particularly for properly cooked garbage. However, during hot weather, it may be advisable to reduce the time of test validity to 3 days. It is, of course, best to subject garbage to field test as soon after cooking as possible. Proof for these conclusions is given by Tables 5 through 9. Any sign of positive reaction to the field test either from incomplete cooking or "recontamination" is objectionable and certain evidence that further cooking or re-cooking is required.

4. Because of the possibility of contamination by acid phosphomonoesterase from pig feces, old garbage and other sources, it is not advisable to attempt to extend the field test to sites other than the original cooker. Tables 6 through 8 give ample evidence for this conclusion.

The field test is simple, rapid and reliable and can be successfully applied in the field for determining proper heat treatment of garbage. It is very important that the field worker perform the test to the letter of the instructions and with full appreciation of the given precautions.

The test is as fast as the present thermocouple probe method, which is used during cooking operations. It has the advantage that it may be used when (a) the
livestock inspector cannot be present during garbage cooking, (b) there is any doubt regarding completeness of cooking as indicated by the temperature gauge probe, (c) there is doubt that all contents of garbage, particularly large scraps, have been thoroughly cooked throughout.

The field test should find wide application for detecting proper cooking of hog swill and may be recommended as a substitute or an adjunct to present methods.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the diligent field work of W. Allen Smith and John T. Sklanka. The support and advice of Drs. Milton J. Tillery and Don Bowers, Swine Diseases Staff, Animal Health Division, Hyattsville, Maryland, is also gratefully acknowledged, as is the assistance of Dr. O. J. Hummon, Technical Services, Beltsville, Maryland, in the design and assembly of field kit materials.
TABLE I. SOME COMMUNICABLE DISEASE AGENTS AND THEIR INACTIVATION HEAT TREATMENT.

<table>
<thead>
<tr>
<th>Disease Agent</th>
<th>Inactivation requirements</th>
<th>Heat exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hog cholera virus</td>
<td>(1) 147</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>or (2) 156</td>
<td>momentary</td>
</tr>
<tr>
<td>Vesicular exanthema virus</td>
<td>(1) 147</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>or (2) 156</td>
<td>momentary</td>
</tr>
<tr>
<td>Foot-and-Mouth disease virus</td>
<td>(1) 147</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>or (2) 156</td>
<td>momentary</td>
</tr>
<tr>
<td>African swine disease virus</td>
<td>(1) 158</td>
<td>10 min.</td>
</tr>
<tr>
<td></td>
<td>or (2) 177</td>
<td>3 min.</td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>137</td>
<td>momentary</td>
</tr>
<tr>
<td>Erysipelothrix rhusiopathiae</td>
<td>(1) 137</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>or (2) flash pasteurization: 161</td>
<td>15 sec.</td>
</tr>
<tr>
<td>Tubercle bacilli</td>
<td>(1) 145</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>or (2) flash pasteurization: 161</td>
<td>15 sec.</td>
</tr>
<tr>
<td>Other (or general)</td>
<td>170</td>
<td>30 min.</td>
</tr>
</tbody>
</table>
Fig. 1. – Illustration of materials required for the field test.

The kit consists of one carrying case containing: 3 solutions; (a) distilled water in a 2 oz. flint glass bottle with dropper calibrated at ½ ml, (b) PMP Solution in a ¼ oz. amber glass bottle with dropper calibrated at ½ ml, (c) Color Producer in a 1 oz. flint glass bottle with dropper calibrated at 1 ml, and (1) a thermostatically controlled incubation block with 4 holes for test tubes, center hole for thermometer and an extension cord and plug for 12-volt automobile cigarette lighter outlet, (2) a separate styrofoam cold-box for PMP Solution, (3) a supply of clean and dry test tubes, 10 x 75 mm, (4) one thermometer 0 to 212° F, (5) a supply of double layer of common cheesecloth, 1’ x 1’, (6) disposable gloves, (7) sterile disposable syringes, 2 ml, for sampling garbage exudate, and (8) clean disposable paper cups to catch garbage exudate.
### TABLE 2.
FIELD AND LABORATORY TEST RESULTS ON HOG SWILL SAMPLES TAKEN BEFORE AND AFTER COOKING

<table>
<thead>
<tr>
<th>LAB. SAMPLE NO.</th>
<th>GARBAGE SOURCE</th>
<th>TYPE OF COOKER</th>
<th>COOKER TEMPERATURE READINGS (°F)</th>
<th>SITE OF SAMPLE IN COOKER</th>
<th>TIME FROM COOKING TO FIELD TEST</th>
<th>FIELD RESULTS</th>
<th>LABORATORY RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 institutions</td>
<td>steam</td>
<td><strong>N.D.</strong></td>
<td>top</td>
<td>before cooking</td>
<td>0</td>
<td>pos. (++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>24 school</td>
<td>direct fire</td>
<td>N.D.</td>
<td>mixed</td>
<td>before cooking</td>
<td>0</td>
<td>pos. (++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>30 institution</td>
<td>direct fire</td>
<td>130 to 140</td>
<td>mixed</td>
<td>during cooking</td>
<td>0</td>
<td>pos. (++)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>26 school</td>
<td>direct fire</td>
<td>N.D.</td>
<td>mixed</td>
<td>before cooking</td>
<td>5 hrs.</td>
<td>pos. (++)</td>
<td>neg.</td>
</tr>
<tr>
<td>*33 military base</td>
<td>direct fire</td>
<td>N.D.</td>
<td>sample from swill can</td>
<td>not cooked</td>
<td>2 days after cook</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
</tbody>
</table>

*Note: The second sample was taken 2 days after cooking; the highest cook temperature attained was not determined.

**Note: N.D. = Not Determined.

Legend:  
(++++) = intense red, equivalent to a raw garbage  
(+++) = medium red, equivalent to semi-cooked garbage  
(+) = slight red, equivalent to almost completely cooked garbage  
neg. = no red, garbage probably properly cooked
### TABLE 3.
FIELD AND LABORATORY TEST RESULTS ON HOG SWILL SAMPLES TAKEN DURING VARIOUS STAGES OF COOKING.

<table>
<thead>
<tr>
<th>LAB. No.</th>
<th>GARBAGE SOURCE</th>
<th>TYPE OF COOKER</th>
<th>COOKER TEMPERATURE READINGS (°F)</th>
<th>SITE OF SAMPLE IN COOKER</th>
<th>SAMPLE AT STAGE OF COOKING</th>
<th>FIELD RESULTS</th>
<th>LABORATORY RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>school</td>
<td>direct fire</td>
<td>uncooked</td>
<td>top</td>
<td>not cooked</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>49</td>
<td>reformatory</td>
<td>steam</td>
<td>uncooked</td>
<td>mixed</td>
<td>not cooked</td>
<td>pos. (+++)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>11</td>
<td>municipality</td>
<td>steam</td>
<td>top - 140 bottom - 170</td>
<td>top</td>
<td>at start of cooking</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>38</td>
<td>municipality</td>
<td>steam</td>
<td>180 to 210</td>
<td>top</td>
<td>during cook</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>40</td>
<td>hospital</td>
<td>direct fire</td>
<td>210</td>
<td>top</td>
<td>during cook</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>46</td>
<td>cafeteria</td>
<td>direct fire</td>
<td>205 to 210</td>
<td>mixed</td>
<td>during cook</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>52</td>
<td>restaurant</td>
<td>direct fire</td>
<td>210</td>
<td>top</td>
<td>during cook</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>13</td>
<td>restaurant</td>
<td>direct fire</td>
<td>210</td>
<td>mixed</td>
<td>during cook</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>6</td>
<td>reformatory</td>
<td>steam</td>
<td>180</td>
<td>middle</td>
<td>after cook</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>*50</td>
<td>poultry plant</td>
<td>direct fire</td>
<td>130</td>
<td>middle</td>
<td>1 day after cooking</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
</tbody>
</table>

*Note: Sample temperature was taken during cool-off, 1 day after cooking; the highest cook temperature attained was not determined.

Legend: cf. Table 2
## Table 4.

Field and laboratory test results taken on random hog swill samples. Cooker temperature readings were either not taken or were not available. Samples are listed according to sampling site for convenience.

<table>
<thead>
<tr>
<th>Lab. Sample No.</th>
<th>Garbage Source</th>
<th>Type of Cooker</th>
<th>Site of Sampling in Cooker</th>
<th>Time from Cooking to Field Test</th>
<th>Field Results</th>
<th>Laboratory Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>restaurant</td>
<td>direct fire</td>
<td>top</td>
<td>1 day</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>25</td>
<td>restaurant</td>
<td>direct fire</td>
<td>top</td>
<td>1 day</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>32</td>
<td>hotel</td>
<td>direct fire</td>
<td>top</td>
<td>5 days</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>35</td>
<td>restaurant</td>
<td>direct fire</td>
<td>top</td>
<td>18 hrs.</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>37</td>
<td>hospital</td>
<td>direct fire</td>
<td>top</td>
<td>2 days</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>39</td>
<td>military</td>
<td>direct fire</td>
<td>top</td>
<td>2 days</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>48</td>
<td>municipality</td>
<td>steam</td>
<td>top</td>
<td>5 days</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>51</td>
<td>officer’s club</td>
<td>direct fire</td>
<td>top</td>
<td>2 days</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>1</td>
<td>cafeteria</td>
<td>direct fire</td>
<td>middle</td>
<td>2 days</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>10</td>
<td>restaurant</td>
<td>direct fire</td>
<td>middle</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>12</td>
<td>military base</td>
<td>direct fire</td>
<td>middle</td>
<td>18 hrs.</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>8</td>
<td>military base</td>
<td>direct fire</td>
<td>bottom</td>
<td>1 day</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>31</td>
<td>air base</td>
<td>direct fire</td>
<td>bottom</td>
<td>not known</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>14</td>
<td>restaurant</td>
<td>steam</td>
<td>mixed</td>
<td>2 days</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>18</td>
<td>restaurant</td>
<td>direct fire</td>
<td>mixed</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>19</td>
<td>restaurant</td>
<td>direct fire</td>
<td>mixed</td>
<td>1 day</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>27</td>
<td>unknown</td>
<td>direct fire</td>
<td>mixed</td>
<td>18 hrs.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>28</td>
<td>restaurant</td>
<td>direct fire</td>
<td>mixed</td>
<td>14 hrs.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>47</td>
<td>restaurant</td>
<td>direct fire</td>
<td>mixed</td>
<td>not known</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
</tbody>
</table>

Legend: cf. Table 2.
### TABLE 5.
RESULTS OF FIELD TESTS PERFORMED ON NEGATIVE (i.e., COOKED) GARBAGE SAMPLES AFTER 24 HRS.
SOME DUPLICATE SAMPLES WERE COVERED DURING THE 24 HR. PERIOD.

<table>
<thead>
<tr>
<th>LAB. SAMPLE No.</th>
<th>GARBAGE SOURCE</th>
<th>TYPE OF COOKER</th>
<th>TEMPERATURE READINGS (°F)</th>
<th>SITE OF SAMPLE IN COOKER</th>
<th>TIME FROM COOKING TO INITIAL FIELD TEST</th>
<th>FIELD RESULTS IN 24 HRS.</th>
<th>LABORATORY RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>military direct fire</td>
<td>200 (ave.)</td>
<td>middle</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>hotel direct fire</td>
<td>200 (ave.)</td>
<td>middle</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>military direct fire</td>
<td>200</td>
<td>middle</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>restaurant direct fire</td>
<td>210</td>
<td>middle</td>
<td>0</td>
<td>neg. (open)</td>
<td>neg. (open)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>municipality steam direct fire</td>
<td>200 (ave.)</td>
<td>middle</td>
<td>0</td>
<td>neg. (covered)</td>
<td>neg. (covered)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>restaurant direct fire</td>
<td>210</td>
<td>mixed</td>
<td>8 hrs.</td>
<td>neg. (open)</td>
<td>neg. (open)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>restaurant direct fire</td>
<td>215</td>
<td>mixed</td>
<td>8 hrs.</td>
<td>neg. (covered)</td>
<td>neg. (covered)</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>municipality steam direct fire</td>
<td>200 (ave.)</td>
<td>top</td>
<td>5 hrs.</td>
<td>neg. (open)</td>
<td>neg. (open)</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>hotel direct fire</td>
<td>215-200</td>
<td>top</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>hotel direct fire</td>
<td>205 (ave.)</td>
<td>top</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>military direct fire</td>
<td>215</td>
<td>top</td>
<td>0</td>
<td>neg.</td>
<td>neg.</td>
<td></td>
</tr>
</tbody>
</table>

Legend: cf Table 2.
TABLE 6.
RESULTS OF FIELD TESTS PERFORMED ON NEGATIVE (i.e., COOKED) GARBAGE SAMPLES TO DETERMINE WHEN THEY WOULD TEST POSITIVE (i.e., INDICATE "RECONTAMINATION").

<table>
<thead>
<tr>
<th>LAB. SAMPLE No.</th>
<th>PERTINENT DATA AND REMARKS</th>
<th>DAYS FROM COOKING TO FIELD TEST</th>
<th>FIELD RESULTS</th>
<th>LABORATORY RESULTS</th>
</tr>
</thead>
</table>
| 23              | Cook temperature: > 200°F
                 | Garbage source: Restaurant
                 | Site of sample: Mixed direct fire cooker.
                 | Remarks: Sample kept uncovered in back yard. Weather hot. | 0          | neg.             | neg.             |
| 41              | Cook temperature: 210°F
                 | Garbage source: Restaurants
                 | Site of sample: Mixed direct fire cooker.
                 | Remarks: Sample kept uncovered in back yard. Weather cool. | ¾          | neg.             | neg.             |
| 29              | Cook temperature: 180 to 215°F
                 | Garbage source: Municipality
                 | Site of sample: Mixed steam cooker.
                 | Remarks: Sample held uncovered in back yard. Weather warm (ca. 75°F high). Rain diluted 4th day sampling. | 0          | neg.             | neg.             |
| 57              | Cook temperature: 210°F
                 | Garbage source: Correctional camp
                 | Site of sample: Top of direct fire cooker
                 | Remarks: Sample open to snow and rain. Weather cold (20°F to low 40°F) | 0          | neg.             | neg.             |

Legend: cf. Table 2.
<table>
<thead>
<tr>
<th>LAB. SAMPLE No.</th>
<th>GARBAGE SOURCE</th>
<th>TYPE OF COOKER</th>
<th>SITE OF GARBAGE SAMPLING</th>
<th>TIME FROM COOKING TO FIELD TEST</th>
<th>FIELD RESULTS</th>
<th>LABORATORY RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>municipality</td>
<td>steam</td>
<td>concrete hog pen</td>
<td>1 day</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>15</td>
<td>prison</td>
<td>steam</td>
<td>concrete feed trough</td>
<td>1 day</td>
<td>pos. (+++)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>36</td>
<td>restaurant</td>
<td>direct fire</td>
<td>feeder 10 min. after dumping</td>
<td>6 hrs.</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td>44</td>
<td>restaurant</td>
<td>direct fire</td>
<td>pail near feeder</td>
<td>not known</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>45</td>
<td>military</td>
<td>direct fire</td>
<td>feeder</td>
<td>2 days</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
</tbody>
</table>

Legend: cf. Table 2
TABLE 8.

RESULTS OF FIELD TESTS OF HOG FECES AND FECES CONTAMINATED GARBAGE.

<table>
<thead>
<tr>
<th>DESCRIPTION OF SAMPLE</th>
<th>FIELD TEST RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces with equal volume of water added</td>
<td>very pos., &gt; (+++)</td>
</tr>
<tr>
<td>½ Feces + ½ Cooked Garbage with equal volume of water added</td>
<td>very pos., &gt; (+++)</td>
</tr>
<tr>
<td>¼ Feces + ¾ Cooked Garbage</td>
<td>very pos., &gt; (+++)</td>
</tr>
<tr>
<td>½ Manure (from pen, including straw) + ½ Cooked Garbage</td>
<td>pos. (+)</td>
</tr>
</tbody>
</table>

Legend: cf. Table 2
### TABLE 9.
RESULTS OF FIELD TESTS PERFORMED ON NEGATIVE (i.e., COOKED) GARBAGE AFTER DUMPING INTO HOG FEEDING TROUGH.

<table>
<thead>
<tr>
<th>LAB. SAMPLE No.</th>
<th>PERTINENT DATA &amp; REMARKS</th>
<th>TIME FROM COOKING TO FIELD TEST</th>
<th>TIME AFTER DUMPING TO FIELD TEST</th>
<th>FIELD RESULTS</th>
<th>LABORATORY RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>Cook temperature: 180°F (ave.)</td>
<td>0</td>
<td>1 hr.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td></td>
<td>Garbage source: Municipality and prison</td>
<td>12 1/2 hrs.</td>
<td>1 hr.</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td></td>
<td>Sampling: 3 sites in feeder from steam cooker</td>
<td>12 1/2 hrs.</td>
<td>1 hr.</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td></td>
<td>Remarks: Feeder contained some old garbage and much water. Samples were taken at 3 locations in feeder. Weather cold ( &lt; 35°F).</td>
<td>14 1/2 hrs.</td>
<td>3 hrs.</td>
<td>pos. (+++)</td>
<td>pos. (+++)</td>
</tr>
<tr>
<td>59</td>
<td>Cook temperature: 195 to 200°F.</td>
<td>0</td>
<td>1 hr.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td></td>
<td>Garbage source: Municipality</td>
<td></td>
<td>2 hrs.</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td></td>
<td>Sampling: Various sites in feeder from steam cooker</td>
<td></td>
<td>3 hrs.</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
<tr>
<td></td>
<td>Remarks: Garbage was dumped into clean feeders. Weather cold ( &lt; 45°F).</td>
<td></td>
<td>5 hrs.</td>
<td>pos. (+)</td>
<td>pos. (+)</td>
</tr>
</tbody>
</table>

Legend: cf. Table 2.
REFERENCES

Your committee recognizes the progress in hog cholera eradication made during the past year. State and Federal actions have, for all practical purposes, eliminated the use of hog cholera vaccines. All but three states are engaged in final "stamping out" actions against this disease.

Your committee wishes to emphasize that the cooperative State-Federal effort to eradicate hog cholera is entering a crucial stage and that the total commitment of all involved is necessary to achieve the goal of a "hog cholera free" United States. Your committee is pleased to note the many representatives of the various segments of the swine industry present at this meeting who have involved themselves in this effort.

Because of the urgency of hog cholera eradication efforts, a special meeting of this committee was held on June 13, 1969, in Columbus, Ohio, to explore means of intensifying eradication program procedures, particularly in regard to incorporating market standards into the program standards. A number of recommendations were made at this meeting and later adopted by this Association. However, your committee is distressed with the lack of reaction to these recommendations. Your committee believes that the adoption of these recommendations requires considerable effort, and remains concerned that they have not been fully implemented.

As predicted, some states experienced an increase in the reported number of hog cholera outbreaks following discontinuance of the use of hog cholera vaccines. This is particularly true among swine in trade channels. The majority of states, however, have not experienced an increase in outbreaks. Your committee recognizes that improvement in market standards is necessary to prevent further increases in incidence of this disease.

Recognizing that a large number of outbreaks in recent months have been associated with movement of feeder pigs, both intrastate and interstate, your committee has given particular attention to this area.

Your committee notes that Federal regulations cannot deal with the intrastate spread of hog cholera, which accounted for 41 percent of the hog cholera outbreaks in FY 1969. Therefore, your committee recommends that the following addition be made to the standards for the cooperative State-Federal Hog Cholera Eradication Program without delay:

Wherever pigs are assembled for sale or shipment (either interstate or intrastate), they must be in compliance with the marketing standards established by this Association; otherwise, they will not be permitted to move interstate.
standards would include:

1. Individual identification of feeder pigs or breeding swine to the farm-of-origin and maintenance of this identification to the premises of destination.

2. Movement of feeder pigs and breeding swine limited to one market only, except that movement through a maximum of two markets would be permitted if the ownership of the swine remains the same through the two markets. (This would provide for movement from farm-of-origin through market No. 1 and market No. 2 to farm-of-destination; provided, the ownership remained the same from the first market to the farm-of-destination.)

Your committee also recommended that “1” and “2” preceding be included as part of proposed Federal regulations on interstate shipment.

It was recognized by your committee that the procedure described in “2” preceding is not the optimum in disease control and, therefore, represents a compromise between what is best and what is practical. Therefore, it was noted that if this procedure does not prove satisfactory in halting the spread of hog cholera, it should be reevaluated by your committee and appropriate action taken.

Your committee also heard discussions of other proposed Federal regulations and made the following recommendations in regard to these:

States should take action to extend to 30 days the quarantine period for imported feeder pigs and breeding swine, but until such time as most states have adopted such quarantine provision, Federal regulations should retain the 21-day provision for quarantine.

Serum dosage for pigs moving through markets or assembly points should be increased to the following:

<table>
<thead>
<tr>
<th>Weight of Pig</th>
<th>Serum Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs less than 20 pounds</td>
<td>20 cc. serum</td>
</tr>
<tr>
<td>20- to 40-pound pigs</td>
<td>30 cc. serum</td>
</tr>
<tr>
<td>40- to 90-pound pigs</td>
<td>35 cc. serum</td>
</tr>
<tr>
<td>90- to 120-pound pigs</td>
<td>45 cc. serum</td>
</tr>
<tr>
<td>120- to 150-pound pigs</td>
<td>55 cc. serum</td>
</tr>
<tr>
<td>150- to 180-pound pigs</td>
<td>65 cc. serum</td>
</tr>
<tr>
<td>Pigs over 180 pounds</td>
<td>75 cc. serum</td>
</tr>
</tbody>
</table>

(or half the above amounts of antibody concentrate)

In connection with this recommendation, your committee notes that the use of serum is an interim measure only to be used during the period necessary to implement the market standards recommended at the June meeting, those recommended under “1” and “2” preceding, and for so long as the level of exposure at markets presents a threat to pigs moving in trade channels. In addition, the continued use of serum should be reevaluated at frequent intervals.

Your committee further recommends that the various states give recognition to Phase IV and “hog cholera free” states by relaxing their requirements for serum treatment of pigs moving directly to farms of destination from approved markets in
the Phase IV and "hog cholera free" states.

Your committee recommends that Federal regulations be amended to prohibit the interstate shipment of swine which have been fed raw garbage, and to restrict the interstate shipment of swine which have been fed cooked garbage, to immediate slaughter only.

Your committee heard a report on a promising and practical test to determine whether garbage fed to swine has been properly cooked, and recommends this test be adopted.

Your committee also recommends that Federal regulations be amended to prohibit the interstate shipment of all swine exposed to hog cholera, except for immediate slaughter for special processing. Such interstate shipments are now permitted from Phase III states to permit salvage of apparently healthy swine from herds infected with hog cholera.

Your committee recommends that the Federal government assume 100 percent of indemnity costs in disposing of hog cholera-infected swine involved in interstate commerce when a meaningful quarantine has been applied by the state of destination, and hog cholera occurs within the quarantine period. This 100 percent Federal indemnity should be restricted to those animals directly involved in the interstate shipment.

Your committee has reviewed the possible use of bovine virus diarrhea (BVD) vaccine in this eradication program. Proposals for its use and further evaluation were rejected.

Your committee recommends that the provision be retained which requires that a period of at least 12 months shall have passed without hog cholera being diagnosed before a state can be declared "hog cholera free". In the event a "hog cholera free" state loses that status, your committee recommends that the minimum time period for regaining "hog cholera free" status be reduced from 12 months to 6 months. Your committee further recommends that a period of 3 months should elapse without hog cholera being diagnosed in order for a state to be eligible to advance from Phase III to Phase IV.
EVALUATION OF A COMPARATIVE TEST
FOR TUBERCULIN REACTORS
FROM A NO-GROSS-LESION HERD

Thomas H. Vardaman, D.V.M., M.S.¹
and
Aubrey B. Larsen, D.V.M., M.S.²

INTRODUCTION

The Committee on Tuberculosis of the United States Animal Health
Association has repeatedly called attention to the problem of “no gross lesion”
reactors and pointed out the need for further study in this area.

Berman et al.¹ reported results of a survey carried out in Wisconsin to
determine the cause of sensitivity to tuberculin in cattle that did not appear to be
infected with Mycobacterium bovis as determined by postmortem examinations.
They obtained cultures of mycobacteria from 14 cattle out of a total of 172
tuberculin reactor cattle. Two cultures resembled M. avium; one resembled M. bovis;
the others were not identified but apparently belonged to unclassified groups.

This investigation concerned the diagnostic value of a comparative test using
mammalian tuberculin and johnin. The purpose was to determine the cause of
tuberculin hypersensitivity in cattle apparently not infected with M. bovis.

MATERIALS AND METHODS

Selected cattle from a herd of 200 were used. Although some cattle from this
herd had reacted to intradermal injection of either tuberculin or johnin or both in
the past 10 years, there was no clinical or gross postmortem evidence of
tuberculosis or paratuberculosis. No evidence of tuberculosis had been observed in
the caretakers. Skin lesions were not observed in any cattle.

The herd was tested with 0.1 ml of mammalian tuberculin in the caudal fold.
Reactions of P¹, P², and P³ occurred in 20 cattle 72 hours later. These 20 were
selected for comparative testing using ARS mammalian tuberculin and johnin from
current lots being used by the Animal Health Division, ARS, for field testing.

The johnin was injected on one side of the neck and the tuberculin in the
identical area on the opposite side. The hair was clipped from each injection site.
Each cow was given 0.1 ml of tuberculin and 0.2 ml of johnin. The skin thickness
was measured before each injection and after 48 hours. The increase was recorded
as the size of the reaction.

After completing the tests the 20 cattle were killed and examined. Bacteriolog-
cal cultures were made from the following tissues: mandibular, cervical, thoracic,
mesenteric and prescapular lymph nodes; lungs and Peyer’s patches. The tissues

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²National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricul-
were processed separately for culturing by a previously described method,\(^3\) and inoculated onto the following mediums; (1) Dubos medium with albumin complex added, (2) Middlebrook 7H10 medium, albumin complex added, and (3) Louenstein's medium with eggs.

Samples of the individual tissue digests from each animal were pooled to form a composite inoculum from each animal into 2 rabbits, 2 chickens, 5 guinea pigs and 6 mice. Six weeks after inoculation all laboratory animals were tuberculin-tested and 1 rabbit, 1 chicken, 2 guinea pigs and 3 mice from each group were examined postmortem for evidence of tuberculosis. After an additional 6 weeks the remaining laboratory animals were again tuberculin tested and then examined postmortem.

Smears of the ileocecal valve were examined microscopically for small acid-fast organisms typical of \textit{Mycobacterium paratuberculosis}.

**RESULTS**

Four cattle developed swellings greater than 5 mm to tuberculin and less than 5 mm to johnin, while 6 cattle developed swellings greater than 5 mm to both tuberculin and johnin. The remaining 10 showed smaller reactions to both products, however, the trend was larger responses to tuberculin than to johnin (Table I).

Acid-fast bacilli indistinguishable from \textit{Mycobacterium paratuberculosis} were in tissues of the ileocecal valves from 3 cattle.

Visible lesions typical of tuberculosis were not observed at postmortem examination. Mycobacteria were not isolated from tissues of 16 cattle. Cultures of mycobacteria, unclassified Runyon group III were isolated from each of 3 cattle and 1 culture (considered to be a Runyon group IV) was isolated from another (Table I);* none of these 4 isolants was identified further.

None of the chickens inoculated with the processed tissues responded to avian tuberculin and no lesions were observed on postmortem examination. Several inoculated rabbits, guinea pigs and mice had small reactions to mammalian tuberculin. No lesions resembling tuberculosis were in any laboratory animals on postmortem examination.

**DISCUSSION**

The responses to mammalian tuberculin observed in this herd apparently did not result from \textit{M. bovis} infection. Comparative testing with johnin and mammalian tuberculin did not help clarify the cause of nonspecific responses to mammalian tuberculin routinely used in the bovine tuberculosis eradication program. We did not use avian tuberculin because our experience has shown avian tuberculin to be less sensitive than johnin for detecting cattle infected with either \textit{M. paratuberculosis} or \textit{M. avium}.

Since unclassified types were isolated from 4 cattle in the population under

*Isolated and identified by Dr. V. H. Mallman, Tuberculosis Research Project, Michigan State University, East Lansing, Michigan.
test, possibly the remaining 16 cattle also were sensitized by such organisms. Three cattle probably had a sub-clinical infection with *M. paratuberculosis* but the comparative test did not show this.

Herd such as the one reported here present a special problem; they are apparently free of *M. bovis* infection but our comparative test was not sufficiently precise to differentiate between infection by *M. bovis, M. paratuberculosis* or atypical and saprophytic mycobacteria.

The comparative test procedure recently described by Brown \(^2\) may be a more effective method and should be evaluated in the field, particularly on such cattle as described here.

**SUMMARY**

Twenty tuberculin reactors from a 200-cow herd with no history of tuberculosis or skin lesions were tested in the cervical region with a comparative test using mammalian tuberculin and johnin and then killed. Selected tissues were obtained at postmortem examination, examined microscopically, and cultured for mycobacteria. Portions of the inoculums from each animal were inoculated into chickens, rabbits, guinea pigs and mice.

Four cattle responded to the comparative test with a 5.0 mm or more increase to tuberculin and less than 5.0 mm increase to johnin. Six cattle responded with a 5.0 mm or more increase to both tuberculin and johnin. The remaining 10 showed smaller responses to both products, however, the trend was larger responses to tuberculin than to johnin. No evidence of tuberculosis was observed in any cattle or laboratory animals on postmortem. Cultures of mycobacteria (unclassified Runyon groups) were obtained from the tissues of 4 cattle. Acid-fast bacteria indistinguishable from *Mycobacterium paratuberculosis* were found on microscopic examination of the intestinal tissues of 3 cattle. It was concluded that the comparative test employed on these cattle was not an effective method of arriving at a correct diagnosis and that a better test is needed.
<table>
<thead>
<tr>
<th>Cow</th>
<th>Cervical Skin Test*</th>
<th>Mammalian Tuberculin</th>
<th>Johnin</th>
<th>Isolation</th>
<th>Tissue from which isolation was made</th>
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<td>4355</td>
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</tbody>
</table>

*Skin test response measured in mm of increase over normal skin thickness.
†M. paratuberculosis in intestinal tract.
+Not applicable.
REFERENCES


THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

by
A. F. Ranney,1 D.V.M., M.S.

Presented at the Seventy-third Annual Meeting
United States Animal Health Association
Milwaukee, Wisconsin
October 17, 1969

For many years man talked about going to the moon. This past year man reached that objective. Skeptics of rocketry and space adventure said, "I won't be around to see it happen." And, they said, "It'll never happen." "Why bother?" Nevertheless, man had the capacity and he applied it. He had an objective clearly visible to the naked eye; something he could shoot for. Skeptics of the early eradication program for bovine tuberculosis said, "A program is being foisted which cannot be executed." The pioneers in the program were confident it could be done. They knew man had the capacity. Few of the early pioneers in tuberculosis work are around today. All of us would like to be around to see the objective reached. It will, of course, be history!

What will we see when the objective is reached? We will see a slate wiped clean. We know the end we seek is zero. There will be no tickertape parade. Will this accomplishment be remembered as "That's one small step for man, one giant leap for mankind?"

We could review ancient history and wonder about the source of tuberculosis found in Egyptian mummies. But, let's just go back sixty years and take a look at this lad, a victim of the disease. (Figure 1.) In 1921 data showed that 18.2 percent to 26 percent of the deaths from tuberculosis in children were caused by the bovine bacillus.2 We know the source that caused an afflicted lad like this, who was not an uncommon sight in those days. We can be thankful he is an uncommon sight today.

Howard R. Smith3 in his story, "The Conquest of Bovine Tuberculosis in the United States," relates that before 1906 there existed merely a superficial system of post mortem inspection. The records did not give complete information as to the prevalence of the disease. In June of 1906 President Theodore Roosevelt signed into law more thorough and exacting regulations. It will be noted that they were designed as a public health measure to give full protection to all consumers of meat but they did more, in that they gave a true picture of the prevalence of disease observable in slaughtered animals. At the beginning of the State-Federal cooperative program in 1917, enough cattle and hogs were condemned for tuberculosis annually to fill a stock train twenty-two miles long. With this number of cattle and hogs

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being consigned to the rendering tanks for inedible grease and fertilizer, the future of our livestock industry was seriously threatened.

Now, let's move up about 20 years, to the year 1926, and see what the status of bovine tuberculosis was then. (Figure 2.) Over two percent of the total cattle population was tuberculous. Almost a fourth, or 24 percent, of the disease was concentrated in 4 percent of the cattle population. You can see there was a big job to be done.

Dr. Lochead the very next year said, "Bovine tuberculosis is an enormous economic agricultural problem that can be proven by figures and dollars, but it is also a real public health problem that cannot be so definitely proven by figures; and the loss of life and suffering from crippled children through its transmission to humans cannot be so well expressed in money values. To my mind, money spent on its prevention while a drain on the public purse, is a proper expenditure, justifiable according to the value we place on human suffering, incapacity and life. That is always higher than actual commercial value, especially if the life belongs to someone near and dear to us and no one knows whom that will apply to tomorrow."

Dr. Lochead further stated: "... a conservative estimate is that 25 percent of tuberculosis in children and 5 percent of all tuberculosis is of the bovine type and occurs in all instances because it was transmitted from cows to humans."

****

"In New York City, ... estimated that 300 children died each year from bovine infection, and there is no reason to believe that New York is worse in this respect than other large cities of our country."

****

"We can take steps to see that tuberculous animals are not a source of infection by being used in the meat and milk supply. Milk should not be used from a tuberculous cow, whether it is to be pasteurized or not."

Now, some forty years later, the position we are in today will be shown. We will note progress. We will keep in mind what has been done, for the past motivates the future; but, we will concentrate more on what remains to be done. We must work today from a low percentage of M. bovis infection in our cattle population to zero. We are measuring a climb up the hill to the summit, the point of eradication. We understand the philosophy of disease eradication, we have the administrative machinery, the leadership, the resources, and, as one of our leaders, Dr. F. J. Mulhern, said: "The fascination that diseases can be eradicated and that we are on the brink of eradicating some of them, one of which is bovine tuberculosis." He further stated that this should capture the attention of all of us. We note that he did not say "imagination" but "attention."

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In fiscal year 1969, 70 herds in the United States were reported with known *M. bovis* infection. (Figure 3.) We can see that there has been a decline in the number of infected herds reported over the past few years. This suggests progress. We can see that these 70 herds were scattered and involved half of the United States and Puerto Rico. (Figure 4.) The data, however, does not tell us how many infected herds are still undetected. Who can guess where the next infected herd will be spotted or how much tuberculosis will be spread to man or other animals before the disease is eliminated from the herd?

Between the years 1963 and 1969 there was a marked decrease in routine testing. (Figure 5.) Please note that area testing has decreased by 63 percent. We can see the difference in the number of herds with tuberculosis infection detected by routine testing and by traceback on this illustration. (Figure 6.) The marked decrease of 63 percent in area testing may be the major factor responsible for this obvious decline in the number of infected herds detected by routine testing.

Last year I pointed out that the development of the market cattle identification and traceback program has thus far failed to compensate for the decline in area testing. This situation still exists. Four years ago, Dr. Victor Beal furnished us data on slaughter inspection surveillance and the probability of finding *M. bovis* infected herds under the animal identification and traceback program. As you may recall, the statistical data showed that as the incidence of the disease decreases, the percentage of slaughter cattle inspected to detect and trace infection must increase. Area testing as performed in the past may continue to decline and be replaced to some degree by selected area testing. To economically lower the incidence of tuberculosis we must constantly seek a means to improve the efficiency and effectiveness of the market cattle identification and traceback program.

This Association's Committee on Tuberculosis has recognized this need for many years. In the 1961 report it was recommended that appropriate livestock sanitary officials take immediate steps to require individual identification of all livestock moving both intrastate and interstate by properly recorded eartags, tattoos, backtag or brand. It was stated that such identification is essential for an efficient trace-back system involving any animal found to be diseased. We appreciate the recent action taken by the Consumer and Marketing Service of the Department of Agriculture which provides for better identification of cattle carcasses until slaughter inspection is completed. We are confident this will strengthen the program.

Let’s look briefly at the degree of infection when first detected in the 70 herds reported for 1969. (Figure 7.) Of the 25 herds found by routine testing almost two-thirds had early infection while about one-third had advanced infection. Through traceback testing, 45 herds were located. Unfortunately, advanced infection existed in more than half. In any disease eradication program early discovery of the disease is of prime importance. Data for the five-year period, 1964 through 1969 inclusive, is even more impressive in support of routine testing than the data for a one year period. (Figure 8.)

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From regular-kill slaughter inspection there were 267 cases which were recorded as indicative of \textit{M. bovis}. Out of these 267 cases, 58 were laboratory confirmed as \textit{M. bovis} and 209 were unconfirmed. Of the confirmed cases 26 were traced to infected herds and 12 of the unconfirmed cases were traced to infected herds. (\textit{Figure 9.}) This data suggests that undue reliance is being placed on the traceback program at its present stage of proficiency. It appears that we have prematurely reduced area testing.

We have 19 herds listed in the "Red Flag" category. (\textit{Figure 10.}) Three of these have been infected for 11 years or more. We can see where these "Red Flag" herds are located and the approximate number of years they have been infected. Progress has been made since fiscal year 1960 when 239 "Red Flag" herds were recorded.

A total of 21 infected herds located in 11 States were destroyed during the past fiscal year because of tuberculosis. (\textit{Figure 11.}) We can see the location of these 21 herds. It will be noted that the number of herds destroyed with State-Federal indemnity payments for reactors and exposed animals has increased each year since 1966. (\textit{Figure 12.}) We note that 10 tuberculous herds were destroyed in 1966 as compared to 21 in 1969.

But, as we talk of all this progress, we still have in mind the question "What remains to be done?" What about those herds with \textit{M. bovis} infection that remain as a means of further spread? We have the challenge of convincing those concerned with this disease that this spread must be prevented. We are so close to eradication, what is the cause of delay? It is of no matter that the incidence is low, we see that the disease does exist. What is to prevent new pockets of infection from developing? Again, we ask, "What remains to be done before we reach our objective?" We are committed to the eradication of bovine tuberculosis. Dr. Charles H. Mayo said in his address to the 28th Annual Meeting of this Association in 1924 that: "The work of the United States Livestock Sanitary Association in the prevention of tuberculosis in animals and man is being welcomed by the American people who fear the disease and are ready to follow the plans prepared by this society for its abasement."

Eight years ago this Association's Committee on Tuberculosis clearly defined the priorities. First on this list was the appeal that we concentrate on the elimination of tuberculosis from "Red Flag" herds and other herds known to be affected with tuberculosis. Let's conscientiously review these priorities and apply our capabilities.

In summary, as we reach toward this National objective, we'll keep in mind the following:

1. Our accepted philosophy: Eradication has no meaning except as an absolute.
2. Our capacity: We are confident bovine tuberculosis can be eradicated.
3. Our challenge: As the disease decreases, our efforts must increase.

What man wants, man will have. Remember our objective is eradication. When we speak of eradication, we speak of an investment which will return dividends. As sure as the moon is in the sky, a disease unopposed will increase in prevalence. A low rate of infection today will be a high rate tomorrow unless we continue to oppose its existence.
Figure 3.

Tuberculosis Eradication

HERDS REPORTED WITH TB INFECTION

Indicative of *M. bovis*

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Herds</th>
</tr>
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<tr>
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<td>213</td>
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<tr>
<td>1965</td>
<td>141</td>
</tr>
<tr>
<td>1967</td>
<td>86</td>
</tr>
<tr>
<td>1969</td>
<td>70</td>
</tr>
</tbody>
</table>
Figure 4.

BOVINE TUBERCULOSIS - 70 CATTLE HERDS

Fiscal Year 1969

Indicative of M. bovis

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
Figure 5.

**Tuberculosis Eradication**

**ROUTINE TESTING**
Comparative Results, 1969 with 1963

**DECREASE**

- **AREA** 63%
- **HERD ACCREDITATION** 28%
- **MILK ORDINANCES** 37%
- **SALE, SHOW, IMPORTS, OTHERS** 9%

U.S. DEPARTMENT OF AGRICULTURE  
ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE

Figure 6.

**Tuberculosis Eradication**

**DETECTING HERDS WITH TB INFECTION**

NO.  
- 200  
- 150  
- 100  
- 50  
- 0  


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ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE
Figure 7.

**Tuberculosis Eradication**

**STATUS OF INFECTION WHEN FIRST DETECTED**

Routine Testing/Traceback

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Advanced</th>
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</thead>
<tbody>
<tr>
<td>Routine</td>
<td>64%</td>
<td>36%</td>
</tr>
<tr>
<td>Traceback</td>
<td>58%</td>
<td>42%</td>
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</table>

ROUTINE (25 herds)  TRACEBACK (45 herds)

TOTAL 70 HERDS (INDICATIVE OF M. BOVIS) FISCAL YEAR 1969

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Figure 8.

**STATUS OF INFECTION WHEN FIRST DETECTED**

FY'S 1964 THRU 1969

Routine/Traceback

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Advanced</th>
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<tbody>
<tr>
<td>Routine</td>
<td>74%</td>
<td>26%</td>
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<tr>
<td>Traceback</td>
<td>49%</td>
<td>51%</td>
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</table>

ROUTINE 311 TRACEBACK 257

TOTAL HERDS 568 Indicative of M. bovis

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Figure 9.

267 LESION CASES
(Regular Kill) FY 1969

No further infection found

58 Cases
Confirmed
M. bovis

209 Cases
Not
Confirmed
M. bovis

197

12

Traced to infected herds

Data from Meat Inspection (635 Reports) FY 1960

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Figure 10.

Tuberculosis Eradication

'RED FLAG' HERDS (19)

July 1, 1969

9 ▲ Infected 0-5 yrs
7 ★ Infected 6-10 yrs
3 ○ Infected 11 yrs or more

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ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
Figure 11.

Tuberculosis Eradication

21 TB INFECTED HERDS DESTROYED
Fiscal Year 1969

State-Federal Indemnity Paid on Exposed Animals-11 States

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AGRICULTURAL RESEARCH SERVICE
Tuberculosis Eradication

HERDS REPORTED WITH TB INFECTION

Indicative of *M. bovis*

<table>
<thead>
<tr>
<th>Year</th>
<th>Herds Reported</th>
<th>Herds Destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>111</td>
<td>10</td>
</tr>
<tr>
<td>1967</td>
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<td>14</td>
</tr>
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<td>1968</td>
<td>74</td>
<td>16</td>
</tr>
<tr>
<td>1969</td>
<td>70</td>
<td>21</td>
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U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
REPORT OF THE COMMITTEE ON
TUBERCULOSIS AND PARATUBERCULOSIS

Chairman: Grant S. Kaley, Albany, N.Y.

The committee reaffirms that the ultimate goal of the tuberculosis program remains complete eradication of bovine tuberculosis. Eradication means the complete elimination of M. bovis from cattle in the United States so that it does not reappear even in the absence of preventive measure unless introduced from another specie or from outside the United States.

It is apparent to the Committee that variations exist in the eradication program from state to state. It is our opinion that program details such as training and supervision of veterinarians, application of epidemiological principles, enforcement of quarantines, handling of suspects and maintenance of established retest schedules might benefit from critical comparison with the program details of other states in the area or in the country as a whole. It is recommended that the eradication procedures used in each state be evaluated periodically with a view to encouraging maximum compliance with the established uniform methods. It is further suggested that the results of this evaluation be made available to all State and Federal disease control officials. It is recommended that the survey be made by committees established in each of the four USAHA geographical areas. Each committee shall consist of a regional tuberculosis epidemiologist and two Federal veterinarians appointed by the Director of the Animal Health Division, ARS, and three State employed veterinarians appointed by the President of the USAHA, except that in no instance shall a State veterinarian participate in the evaluation of the program in his own state.

It is recommended that a new paragraph (to be known as G) be added to paragraph 2 of the Uniform Methods and Rules for Tuberculosis Eradication. This paragraph shall read as follows:

When the presence of M. bovis has been confirmed by culture on any premises, liquidation of the herd remains the procedure of choice. Otherwise, the herd shall remain in quarantine until it has passed two negative tuberculin tests at intervals of at least 60 days and an additional two negative tuberculin tests at intervals of six months, the total quarantine period to be not less than 16 months after the last reactor has been disclosed. The entire herd must be included in three of the four required tests.

It appears to your committee that it is time to recognize the cooperation and contribution of the livestock industry to the cause of tuberculosis eradication by
easing, to the extent feasible and practicable, the restrictions placed on the
movement of bovine animals because of tuberculosis. It is suggested that this can be
done by reciprocal agreement whereby states that have made similar progress toward
eradication modify their interstate health requirements to permit free movement of
unquarantined cattle without tuberculin test.

The committee discussed in some detail possible substitution of more
descriptive designations in place of the terms “Modified Accredited Area” and
“Accredited Tuberculosis Free Area (M. bovis in cattle)” A committee of two, Dr.
A. F. Ranney and Dr. D. S. Imgraham, was appointed to consider this matter and to
report back to the committee in Philadelphia in 1970. It was the consensus of the
group that further changes in the Uniform Methods and Rules be deferred until the
sub-committee report could be considered.

The committee recommends that each state take active steps to attain legal
authority by July 1, 1973 to order the destruction of any herd infected with bovine
tuberculosis.

This committee has been informed of evidence of tuberculosis in shipments of
cattle from Mexico. It is recommended that the USDA survey the situation to
determine whether or not the movement of Mexican cattle into the United States
represents a hazard to the health of domestic livestock.

This committee wishes to reaffirm its concern for the losses caused by
tuberculosis in swine and poultry. We therefore, recommend the development of an
identification system for market swine and fowl.

Your committee devoted time to the discussion of Johne's Disease problems
with specific reference to the possibility that uniform guidelines for handling
infected herds might be worked out.

A sub-committee, consisting of Drs. A. B. Larsen, W. D. Yoder, and A. R.
McLaughlin, was requested to consider this matter in detail and to report back at
the meeting of the Committee in Philadelphia in 1970.
SURVIVAL OF FOOT-AND-MOUTH DISEASE VIRUS
ON MEAT PACKAGING MATERIALS

P. Gailiunas, G. E. Cottral, and F. W. Scott*

From the Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944.

SUMMARY

Foot-and-mouth disease virus (FMDV) remained infectious for approximately 33 to 398 days on meat packaging materials experimentally contaminated with infected bovine tissues. Packaging materials tested were: cardboard from boxes, wood from barrel staves, and metal from tin cans. The infected tissues included: blood, serum, ground lymph nodes, and tongue epithelium mixed with fat, each of which was spread as thin films on pieces of cardboard, wood, and metal. The contaminated materials were stored at 4 C with an average relative humidity of 85 percent.

Under the experimental conditions, FMDV survived in the infected serum, smeared on cardboard, wood, and metal for 33 to 35 days; in blood for 55 days; in the ground lymph nodes for 57 days; and in fat for 398 days. Virus recovered from the stored specimens was virulent and infectious, as demonstrated by inoculation in steers.

Under actual conditions practiced in the meat packing industry and trade, FMDV probably would survive on meat wrappers much longer than was demonstrated in the present study, since meat is usually stored and shipped either frozen or at temperatures of -1 C to 1 C. Thus, meat wrappers and shipping containers may be as hazardous as are frozen or chilled meats and other raw animal products imported from countries where foot-and-mouth disease (FMD) is enzootic.

INTRODUCTION

The capacity of foot-and-mouth disease virus (FMDV) to survive under the most adverse environmental conditions is the basic reason for the sudden flare-ups of foot-and-mouth disease (FMD) epizootics. Bachrach et al. demonstrated the existence of thermal-resistant wild populations of FMDV and the stability of its ribonucleic core to hydrochloric acid and heat. A small fraction of suspended FMDV retained its infectivity despite heating the suspension at 80 C for 6 hours. According to Nauryzbayev, FMDV present in fragments of guinea pig epithelium is able to resist the destructive action of 3 percent sodium hydroxide and 1.5 percent formaldehyde solutions for as long as 6 hours. It is not surprising, therefore, that under natural conditions where such severe exposures to physical or chemical insults are seldom reached, FMDV is able to survive and preserve its full replicative power for months and, perhaps, several years.

*Present Address: New York State Veterinary College, Cornell University, Ithaca, N.Y.
The data on the stability of FMDV on various objects in the environment are of great epizootiological interest even though they are mostly of a general nature and sometimes lack specific details. According to Magaha, the longest recorded survival of FMDV in the field occurred during the California outbreak of 1924 when virus persisted on a farm for 347 days. During the latest FMD epizootic in England, a reappearance of FMD occurred on some of the restocked premises 41 to 145 days after completion of disinfection. The recrudescence of disease was attributed to contaminated hay, straw, silage, manure, sacks or a trailer, or to a knife used for paring sheep’s feet. Generally, the survival time of FMDV in the field seems to vary considerably depending on the climatic and geographic conditions. Campion and Gatto observed that under the natural conditions in the Argentina FMDV survived 9 days on the corral fencing and 11 days on the ground. Kindyakov reported, however, that in the Kazakhstan province, which is exposed to the Asiatic winds and is a drought area, FMDV survived on contaminated plants and soil up to 15 days in summer and up to 165 days in autumn and winter. Also, in winter, FMDV survived in hay stacks for 185-200 days.

On objects in the immediate vicinity of the host, FMDV was reported to survive on bran and sacks 20 weeks; on flour 7 weeks; in tap water at ambient temperatures 41 days; and on brick and wooden walls 31 days in winter and 12 in spring. According to Hess, FMDV dried on clothing, may survive up to 100 days. Even packages and letters may serve as disseminators of FMDV. Cases were recorded when the disease broke out 4 days after the arrival of mail from an infected region. Fritschi described a case where FMDV was apparently transported in the grooves of tire treads.

Live animals may become carriers of FMDV for a long time following recovery from the disease or after an inapparent infection. The studies reported by Van Bekkum et al. in 1959, and continued in many laboratories thereafter, revealed the occurrence of latent, low-infectivity populations of FMDV, apparently located in the upper respiratory or alimentary tracts of the host, which were able to persist in convalescent animals for as long as 2 years. Epifanov and Shalashov reported in 1968 the isolation of a virulent strain of FMDV, from the erythrocytes of convalescent cattle, up to 460 days postinoculation (DPI). Decades ago, Fortner found infectious FMDV in the blood of convalescent guinea pigs 198 DPI. According to the most recent studies by Cottrial and Bachrach, however, FMDV could not be demonstrated in the blood of cattle longer than 5 DPI. Conceivably, the meat and other animal products derived from carriers of FMDV may serve as a source of contamination of various objects during packing house operations.

The persistence of FMDV in animal products and by-products deserves particular attention. In salt-cured hides, FMDV may survive up to 352 days; 210 days at 1 C in bone marrow; 60 days in lymph nodes; 73 days at 4 C in cattle blood; 70 days in the swine blood; and 19 days in cattle joint fluid. At freezing temperatures, FMDV survives in most organs of swine and calves for as long as 210 days. According to Mohlmann, FMDV remains infectious on cattle hair as long as 4 weeks. Voiven found that cattle hairs contaminated with infectious vesicular fluid retained FMDV for 5 days in summer and 20 days in winter. Virus applied on wool of live sheep loses its virulence within 3-8 days in
summer. However, in the wool bales that are stored at 13-15 C, FMDV may survive up to 30 days.

The review on the survival of FMDV, as presented here, stresses the immense variety of conditions under which the virus may persist for a very long time. Comprehensive reviews and commentaries on FMDV survival in the host and environment were prepared by Röhrer in 1967, and Cottral in 1969. However, the available information is not specific enough to assess the hazard of FMDV survival on meat packaging materials.

The purpose of this study was to determine whether or not, and for what period of time, FMDV would survive on contaminated meat packaging materials that are stored and shipped under the conditions commonly practiced in the meat packing industry and trade.

MATERIALS AND METHODS

**Experimental cattle.** Grade Hereford steers were used to produce infected tissues and verify infectivity of FMDV when recovered by isolating in tissue cultures from infective smears that had been used to contaminate meat packaging materials. The steers were clinically healthy, averaged 300 kg., and ranged in age from 18 to 24 months. Their management, isolation, and handling procedures during experimentation have been described.

**Viruses.** Four strains, representing the three principal types (A, O, and C) of FMDV were used. They were designated: subtype A-61*, strain A-1; subtype O1, strain 0-2; subtype O1/3*, strain 0-9; and subtype C-Rezende, strain C-3. These viruses were isolated from the Argentina field outbreaks in 1961, 1962, and 1963. All strains had been passaged only in cattle. Their history, classification, and the technique for the preparation of virus inoculums were reported by Cottral and elsewhere.

**Tissue cultures.** Primary monolayer tissue cultures from bovine kidney cells were used. Their production in 4-oz prescription and 5-liter Povitsky bottles has been described. The medium for cell growth in prescription bottles was composed of Hanks' balanced salt solution containing 6 percent bovine serum, 0.5 percent lactalbumin hydrolysate and 0.01 percent phenol red (LC medium). The same medium, without serum was used in Povitsky bottle cell cultures.

**Virus assay.** Quantitative and qualitative assays of FMDV in tissue samples, before and after storage, were made in tissue cultures by both the plaque and Povitsky-bottle techniques, and by inoculation of steers. For titration, 0.1 ml of each 10-fold serial dilution was inoculated into 3 prescription-bottle cultures and the titers were calculated per gram of solid, or milliliter of liquid-test materials. When virus concentration was very low, Povitsky-bottle tissue cultures, containing 1,000 ml of LC medium, without serum, were inoculated with respective specimen suspensions ranging from 30 to 50 ml. To confirm infectivity for the natural host, suspensions of selected specimens were inoculated, in 2- to 5-ml amounts, into the lingual mucosa of steers.

**Meat packaging materials.** From a wide array of packaging materials available

---

*Subtype designations (temporary) made by Dr. K. Federer, Pan Am FMD Center, Rio de Janeiro, Brazil.
in the meat packing industry, the cardboard from boxes, wood from barrel staves,
and tin can metal plates were selected for testing the survival time of FMDV on
meat shipping containers. They were chosen on the basis of their representative
properties with respect to the adsorbability, surface texture and moisture retention.
Also, these packaging materials were selected for testing because they meet the
requirements of the variety of meat products as well as methods of merchandising,
and are widely used in bulk packaging and long-distance shipping.

To facilitate the experimentation, all these materials were cut into pieces
approximately 4.5 by 6.5 cm. The cardboard specimens were cut from ordinary
cartons and boxes made by several paper manufacturers. Two types of wood were
used: 1) hardwood (oak) specimens that were cut from the staves of barrels used in
shipping certain imported animal products; and 2) soft wood pieces (pine) that
were taken from the staves of a slack barrel. Metal specimens were cut from the
ends of imported tin cans that had contained roast or corned beef; they were free
of rust and other damages.

To prevent bacterial contamination during testing, the wood and metal pieces
were washed in distilled water several times, dried, and sterilized by heat. The
cardboard, also, was heat-sterilized.

EXPERIMENTAL PROCEDURES

Collection of tissues. — Fifteen steers were inoculated with four strains of
FMDV (Table 1) either by intralingual or intramuscular route and neocropsied at
the peak of viremia. Blood was drawn from the jugular veins before the kill and
stored at -50 C. Serum specimens were obtained by holding the freshly drawn blood
at 4 C for 24 hours; it was then divided into 5-ml portions and frozen at -50 C.

For lymphoid tissue specimens, the right prescapular and the left internal iliac
lymph nodes were removed from the carcass, trimmed of fat and frozen in sterile
containers at -50 C. The source of virus for beef fat specimens was infected bovine
tongue epithelium collected from fresh vesicles. It was placed in sterile screw-
capped vials and stored at -50 C. To simulate the natural slaughtering conditions,
neither heparin, antibiotics, nor any other chemical additives were used during the
collection and processing of blood and other tissue specimens.

Further processing of specimens. — When required, one type of tissue from
each individual steer was processed at a time for use as a contaminant of the meat
packing materials. Each of the respective tissues was thawed by placing the
specimen bottles in a water bath for approximately 2 hours at 24 C. A portion of
the thawed tissues was set aside for virus titration that was done the same day. The
required amount of tissue was weighed, minced, or processed otherwise as specified
later, and smeared in an oval area, 2-4.5 cm, in one milliliter or one-gram amounts
on each of several pieces of the previously prepared cardboard, wood, and metal.

The smears of serum dried rapidly and seemed to be slightly adsorbed into the
cardboard and wooden blocks. After drying for 2 hours at 23 C the smears became
glossy and were surrounded by slight ridges around the edges. When thawed, the
fresh frozen blood separated into a clot and serum. They were ground and mixed in
a pestle into a viscous jam-like mass and smeared individually on the required
number of pieces of the different meat packaging materials. Some smears had small
projecting chunks of blood clots, not exceeding 3 millimeters in diameter.
The lymphoid tissue specimens were prepared by cutting thawed lymph nodes into small cubes and grinding them, with addition of an equal part of LC medium, into a sticky grey mash. The fat specimens were prepared in a similar way, grinding normal uninfected bovine kidney fat (tallow) into a greasy mass and then thoroughly mixing it with ground infectious bovine tongue epithelium, using an epithelium-fat ratio of 1:5. The mixture appeared as a grey paste. Both the lymphoid tissue and fat smears adhered well and became semi-solidified on the surfaces of the test materials.

Storage conditions. — In each experiment, the thawing, grinding, and other operations lasted approximately 3 hours. The drying of smears required an additional 2 hours. All processing was done at 23 °C with relative humidity approximately 30 percent. After drying, the specimens were placed in a large refrigerator box at a constant temperature of 4 °C and relative humidity approximately 82 to 88 percent. A fan provided a continuous air current maintaining an even temperature and humidity in the refrigerator space. Sterility precautions were strictly observed during all operations.

Recovery of virus after storage. — Several smears of each type of tissue derived from one steer were tested in sequence as to the viability of the virus. At selected intervals of storage, specimens were taken out of the refrigerator, the tissue smears scrapped off carefully with a scalpel, and weighed. Then, suspensions were prepared using LC medium according to the procedure described, and assayed for virus in tissue cultures. A portion of the suspension was retained frozen at -50°C for a possible inoculation in steers.

RESULTS

From 69 experimentally infected bovine tissue smears made on meat packaging and shipping materials, 51 were positive for infectious FMDV following prolonged storage in a refrigerator at 4 °C and 82-88 percent average relative humidity.

Under these conditions, FMDV remained infectious in the serum smears for 33 to 35 days. The smears of dried whole blood still contained relatively high quantities of FMDV after 40 to 48 days. Smears of ground lymphoid tissue harbored more than 2 log units of FMDV after storage for 50 to 57 days. Considerable quantities of FMDV persisted in fat smears for as long as 82 and 112 days storage. Trace amounts of FMDV, sufficient to infect steers, were detectable in fat samples through 398 days of storage. Results of the quantitative assays and infectivity tests of FMDV in different bovine tissue smears dried on various materials are given in Table 2. The data on the longest demonstrated survival of FMDV on meat packaging materials is summarized in Table 3.

The 15 infected steers, used as a source of blood and other infectious tissues to prepare smear samples were inoculated with 4 different strains of FMDV (Table 1). The infectivity of FMDV, recovered from smear samples after periodic intervals of storage, was ascertained by steer inoculation. Selected representative samples were inoculated into 18 steers. From 18 samples tested in steers, 15 were positive for FMDV (Table 2). Steers developed typical clinical signs and lesions within 1 to 3 days postinoculation demonstrating the virulence, infectivity and potential hazard of FMDV surviving on materials used in meat packaging and shipping.

No attempt was made during these studies to determine the decay rate or
terminal inactivation point of FMDV under these specific conditions. The limited number of samples indicated, however, that the natural inactivation of FMDV proceeds relatively fast in the dried blood (Table 2). Initial linear decline of the virus titer was also noticed in lymph node smears. The FMDV derived from bovine lingual epithelium and mixed with fat decayed at a very slow rate (Table 2).

Testing indicated that the physical structure and the chemical composition of three different solid materials possessing little or no adsorbability, had no appreciable effects on the decay rate of FMDV smeared on their surface (Table 2). The essential variables determining the decline and final loss of viral infectivity were temperature and relative humidity in the storage chamber, as well as the type of animal tissue used as the vehicle of FMDV.

DISCUSSION

Foot-and-mouth disease virus survives on meat packaging materials and shipping containers much longer than is needed to transport animal products from one continent to another. The usual time required to ship meat from South America and South Africa to England is approximately 21 and 17 days, respectively.48 Since the commercial and hygienic durability of chilled beef is only 35 days, it is obvious that FMDV present on meat wrappers may remain infectious until distribution in the open market. Furthermore, under actual conditions practiced in the meat trade, the survival of FMDV on meat packaging materials, by all probability, is much longer than was shown in this study. While the present experiments were conducted at an intentionally high storage temperature of 4 C, the optimal transportation and storage temperature for chilled meat is -1 to 1 C with a relative humidity of 80-90 percent.24,43,48 It is well known that low temperatures favor the long survival of FMDV.15,19 When beef was stored at 4 C, FMDV persisted in bone marrow 73 days.15 However, when the temperature was lowered to 1 C, the survival time in bone marrow extended to 210 days.14 Similarly, Scott et al.4 showed that in saliva collected from infected cattle FMDV persisted only one day at 37 C, but for 24 days when stored at 23 C. It is most likely that at freezing temperatures, FMDV would survive on meat wrappers for approximately the same time as in frozen meat. This time is given by Savi et al.41 as 210 days. According to Witmann,52 at -15 to -20 C, the concentration of FMDV in the organs and tissues of experimentally infected swine remains relatively constant 98 to 150 days.

Although present tests involved only three different types of meat packaging materials, the results obtained may well be applicable to other articles used in meat wrapping and shipping, like cotton cloth, burlap wrapping, canvas, and crates. The persistence of FMDV on such objects has been recorded in previous reports. In burlap and stockinet, contaminated with infectious blood, FMDV survived at 13 to 30 C for 45 days.8 On rubber boots, contaminated with blood, virus persisted8 for 102 days. According to recent observations by Nauryzbayev,38 FMDV survives on cotton fabrics and rubber footwear for 63 to 68 days in winter (1 to 22 C) and for 23 to 28 days in spring (9 to 22 C). The role of fabrics in spreading infectious agents is, also, well known from the studies of other viruses. Sidwell et al.45 demonstrated that vaccinia virus may survive on contaminated wool blankets for 14 weeks, if held at 25 C with relative humidity of 35 to 78 percent. Thus, the
available data seem to justify the conclusion that practically all materials used in packaging and shipping meats and other raw animal products may act as disseminators of FMDV.

Infected serum and fat seem to be particularly hazardous. Dried serum is barely visible on the surface of cardboard of wood, and fat acts as a protective barrier. As shown in this study, FMDV present in ground tongue epithelium mixed with fat survived up to 398 days. However, in an aqueous suspension FMDV infectivity was lost in 8 months. It is known that relatively little virus is present in fat of experimentally infected swine and cattle. Similarly, as observed by Bell and Moore, only low concentrations of rabies virus can be isolated from the brown fat of naturally infected bats. However, according to Sulkin et al., the brown fat may serve as a site for storage and multiplication of rabies virus. Since rabies virus persists in brown fat of live bats up to 60 DPI, it was suggested that the adipose tissue may provide a mechanism by which certain hosts may serve as reservoirs of viral agents during interepidemic periods. To what extent this suggestion might be applicable to FMDV, has not yet been determined. However, the danger of fat as a protective barrier for FMDV has been well demonstrated by the present experiments.

Huq reported that at 1.7 to 19.4 C, FMDV survived on the cardboard not longer than 2 days, and on the wood 4 days. The much longer virus survival times shown in the present study may be due to improvements in virus isolation techniques. On the other hand, some important conclusions related to the epizootiology of FMDV seem to be just as valid and justified now as they were 4 decades ago: "All these experimental observations on the survival of the virus within and without the animal body help to explain the several ways in which infection may lie dormant for months, and then give rise to further outbreaks of the disease, and it would appear from them that the persistence of the virus in carcasses, or in dried discharges upon fodder, hair, etc., is perhaps of greater epizootiological significance than its carriage in the bodies of recovered animals."

Historical surveys of FMD in the United States and in Europe did not refer to meat packaging materials as a source of virus initiating new FMD epizootics. The latest review by Benyon mentions, however, that 54.2 percent of primary outbreaks that occurred in England between 1954 and 1967, were attributed to the imported meat, bones, and meat wrappers. Thus, field observations recognized the hazards associated with objects involved in meat packaging and shipping. The losses resulting from the latest FMD epizootic in England have been described as "staggering." In reviewing the complexities of problems facing animal disease regulatory officials, Saulmon reminded that FMD and other exotic diseases of animals continue to pose a tremendous threat to the total economy of our country. The information developed in this study should be of value in efforts to prevent the spread of FMDV. It stresses, also, the importance of regulatory provisions governing the supervision and handling of meat wrappers and shipping containers from countries where FMD is enzootic.

ACKNOWLEDGEMENT

The authors acknowledge the technical assistance of Messrs. E. V. Kramer, and H. Mazzaferro Jr., and the clerical assistance of Mrs. J. R. Faller.
TABLE 1
TYPES AND STRAINS OF FOOT-AND-MOUTH DISEASE VIRUS, AND THE NUMBERS OF STEERS USED TO PRODUCE INFECTIOUS TISSUE SMEARS

<table>
<thead>
<tr>
<th>STEER NOS.</th>
<th>VIRUS TYPE AND STRAIN</th>
<th>KILLED DPI*</th>
<th>TISSUES USED FOR SMEARS</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>A-1</td>
<td>3</td>
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</tr>
<tr>
<td>2</td>
<td>A-1</td>
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<td>Blood</td>
</tr>
<tr>
<td>3</td>
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<td>Blood, lymph nodes</td>
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<td>4</td>
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<td>1</td>
<td>Tongue epithelium</td>
</tr>
<tr>
<td>5</td>
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<td>Blood</td>
</tr>
<tr>
<td>6</td>
<td>O-2</td>
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<td>15</td>
<td>C-3</td>
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<td>Tongue epithelium</td>
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</tbody>
</table>

* Days postinoculation
TABLE 2
SURVIVAL OF FOOT-AND-MOUTH DISEASE VIRUS IN REPRESENTATIVE BOVINE TISSUE SMEARS DRIED ON CARDBOARD, WOOD, AND METAL

<table>
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<tr>
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<th>Virus strain</th>
<th>Infected tissue</th>
<th>Initial titer* of virus</th>
<th>Days of storage at 4°C</th>
<th>Titer after storage</th>
<th>Infectivity to cattle**</th>
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*Titer before and after storage, expressed as log10 per ml/ or gm of tissue.

**Infectivity: P. = positive; N - negative; - = not done.
TABLE 3

SUMMARY ON LONGEST DEMONSTRATED SURVIVAL OF FOOT-AND-MOUTH DISEASE VIRUS ON MEAT PACKAGING MATERIALS CONTAMINATED WITH INFECTED BOVINE TISSUES

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<th>INFECTED TISSUE</th>
<th>TYPE OF MATERIAL</th>
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REFERENCES


CLINICAL AND SEROLOGIC RESPONSE
OF AMERICAN WHITE-COLLARED PECCARIES
TO AFRICAN SWINE FEVER,
FOOT-AND-MOUTH DISEASE,
VESICULAR STOMATITIS,
VESICULAR EXANTHEMA OF SWINE,
HOG CHOLERA, AND RINDERPEST VIRUSES

A. H. Dardiri, R. J. Yedloutschnig, and W. D. Taylor

From the Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, Long Island, N.Y. 11944.

The domestic pig (Sus scrofa) in the United States of America is susceptible to African swine fever (ASF), foot-and-mouth disease (FMD), vesicular stomatitis (VS), vesicular exanthema of swine (VES), hog cholera (HC) and rinderpest viruses. The wart hog (Phacochoerus aethiopicus) of Africa, when infected with ASF, does not exhibit frank disease signs but becomes a disease carrier, probably disseminating the virus to susceptible pigs.1

The American white-collared peccary (Tayassu tajucu), like the domestic pig, is of the Suidae family. It ranges in the southern parts of New Mexico, Arizona, Texas2 and southward to Patagonia. The literature lacks information about the peccary's susceptibility to disease agents that are exotic to the swine population in the U.S.A. This work was pursued to investigate the susceptibility of the peccary to ASF, FMD, VS, VES, HC and rinderpest viruses. The findings could be valuable in the control and eradication of these diseases.

MATERIALS AND METHODS

African Swine Fever

Animals. American white-collared peccaries and pigs were used in these experiments. The pecarries were obtained by permission of the Texas Parks and Wild-Life Department from the San Antonio and Houston Zoological Gardens by Dr. Frank Hamilton of the Animal Health Division, U.S. Department of Agriculture. The pigs were of Tamworth or mixed breeds.

Viruses. For animal infectivity, 3 ASF viruses were used: Salamanca virus from Spain, Dakar from Senegal, and Tengani from Malawi. The stock viruses were 1:5 spleen suspension in blood collected from infected pigs. Each infected animal received $10^5$ pig lethal doses.

The Tengani virus, passaged once in pig leukocyte cultures, was used for viral assay in pig leukocyte and bone marrow cultures.

Preparation of leukocyte and bone marrow cultures. Leukocyte cultures were prepared from defibrinated blood obtained from peccaries and pigs by the method of Hess and DeTray.3 Bone marrow was collected from a 3-month-old peccary born in the laboratory and from 2- to 3-month-old pigs. The method used in their
preparation was that described by Malmquist and Hay\textsuperscript{4} and the culture was incubated in Leighton tubes at 37°C for 48 hours.

**Infectivity of cell culture for demonstration of hemadsorption (HA) reaction.** The first cell culture Tengani ASF virus was assayed simultaneously in leukocyte and bone marrow cultures from both animal species. The virus was also assayed in cultures from which the original medium [containing erythrocytes with plasma or Eagle's Minimum Essential Medium (MEM)] was replaced by similar medium containing erythrocytes from the other species. For transposition of erythrocytes, the cultures were washed 3 times with MEM, then overlaid with homologous plasma or medium containing 0.5% erythrocytes from the heterologous species. The cultures were then incubated 24 hours before inoculation with graded virus concentrations.

**Demonstration of inclusion bodies.** Leukocyte and bone marrow cells from peccaries and pigs were cultured in Leighton tubes containing cover slips. Forty-eight and 72 hours after inoculation with ASF virus, the cover slips were removed, stained with Giemsa, mounted on glass slides and examined for inclusion bodies.

**Exposure of peccaries and pigs to ASF virus.** Three groups of animals were housed in separate animal rooms. In the first room, 2 peccaries were inoculated intramuscularly (IM) with the Salamanca virus and 1 pig was given the virus orally. Two other peccaries and 1 pig were added to this group as uninoculated contact controls. In the second room, 1 peccary was inoculated IM with the Dakar virus, 1 pig was given this virus orally and 2 other pigs were not inoculated. In the third room, 6 peccaries were given the Tengani isolate IM and 1 of 2 pigs was given the virus orally. Animals were observed for 60 days. Blood samples were taken from surviving animals 25, 45, and 60 days postinoculation (DPI). Serums were tested for precipitating antibodies by the agar gel diffusion precipitation (AGDP) method.

**Assay for viremia.** Blood specimens were obtained in heparin from the anterior vena cava of the peccaries and pigs at 3, 5, 12, and 25 DPI and were inoculated into pig leukocyte cultures for evidence of HA reaction.

**Testing peccary tissues for ASF virus.** From 1 peccary killed 25 DPI, the following specimens were collected: lymph glands (retropharyngeal, prescapular, mesenteric, inguinal, gastrohepatic, mediastinal, maxillary and popliteal), heart blood, lung, liver, spleen, kidney, thyroid gland, tonsil, testicles, urine, intestine, and feces. A 10% suspension of each specimen was prepared in MEM containing 500 units of penicillin, 500 mcg of streptomycin and 100 units each of mycostatin, polymixin, and kanamycin per ml. Each suspension was inoculated into pig leukocyte cultures for indication of HA reaction. Cultures in which HA was not seen during 5 days were serially subpassaged twice. Also, 1 ml of each suspension was inoculated IM into individual pigs. The pigs were placed in suitable metal cages and observed daily for 25 days before their serums were tested for precipitating antibodies.

**Agar diffusion precipitation test.** The AGDP test was conducted according to the method described by Coggins and Heuschele.\textsuperscript{5} In the tests, kidney tissues were used as antigen with serums from pigs that survived infection and subsequent challenge with Salamanca, Tengani, and Dakar ASF viruses.
**Vesicular Diseases**

The same peccaries used in the ASF infectivity trials were subsequently used to determine their susceptibility to FMD, VS, and VES viruses. The animals were exposed successively to FMD, VS, and VES viruses with intervals of at least 30 days between introduction of each virus.

In each of 2 trials, 6 peccaries which had been exposed previously to ASF virus and 4 domestic pigs which had no ASF experience were used. Four peccaries and 2 pigs were confined to one room; the remaining 2 peccaries and 2 pigs were held in another room.

**Viruses.** FMD, type O\textsubscript{1} (CANEFA -2) virus which had been serially passed 6 times in primary bovine kidney monolayers was inoculated. Stock virus had titers of 106.5 tissue culture infectious doses (TCID\textsubscript{50}) /ml. Indiana type 1 VS virus was used for the first infectivity study and Indiana type 2 was chosen for the second study. Inoculums were 20% suspensions of VS virus-infected chorioallantoic membrane in amnioallantoic fluid.

Similarly, different VES virus types were used in the 2 studies. In the first test was VES, type A48 which had been passaged 12 times in primary swine kidney cultures. For the second study, VES, type E54, also in its 12th passage in primary swine kidney cells was selected. The virus titers were 10\textsuperscript{7} tissue culture 50% infective doses per ml (TCID\textsubscript{50}).

**Animal infectivity.** Suspensions of each of the “vesicular-disease” viruses were inoculated intradermally (ID) into the snout, tongue, and the plantar pad, coronary band, and interdigital space of the right front foot of each subject animal. Only 1 pig and 1 peccary in each room were inoculated.

**Serology.** In the first study, blood for serum samples was collected from the anterior vena cava before exposure or inoculation 22, 24, and 30 DPI with each of the 3 viruses. In the second study, the same method was employed, but bleedings were on the date of inoculation and 16, 21, and 25 days later.

**Virus neutralization tests.** The constant serum varying virus neutralization method was used to determine the level of neutralizing activity of the serum. Neutralizing activity was expressed as virus neutralizing indices (VNI) and calculated by the method of Reed and Meunch.\textsuperscript{6} Serums were tested at a final dilution of 1:10 against viruses used in 10-fold final dilutions. The neutralizing activity of the serums was assayed in 6- to 9-day-old suckling mice, 8-day-old embryonated chicken eggs, and pig kidney (PK 15) cell line cultures for FMD, VS, and VES viruses, respectively.

**Complement-fixation tests.** The technique employed was that described by Stone and DeLay.\textsuperscript{7}

**Agar diffusion precipitation tests.** Convalescent serums were tested for antibodies to FMD virus by diffusing them against the virus infection-associated (VIA) antigen and the method described by Cowan.\textsuperscript{8}

**Hog Cholera**

**Animals.** Six peccaries which had previous experience with the viruses of ASF, FMD, VS, and VES and 3 pigs were utilized in this study.

**Virus.** The hog cholera (HC) virus used to infect the animals was the Ames virulent strain. The inoculum contained 50,000 pig lethal doses per ml.
Exposure of peccaries and pigs to HC. Fifty-four days following exposure to VES virus, the last in the succession of vesicular-disease viruses used, 6 peccaries and 3 pigs were inoculated IM with 1 ml of virulent HC virus. The animals were observed for 30 days.

Rinderpest

Animals. Six peccaries that had been exposed to the viruses of ASF, the 3 vesicular diseases and HC were inoculated IM with rinderpest virus. Three pigs which were unexposed to any of the named viral agents were also utilized. Of these pigs, 2 were held in a room apart from the peccaries and were inoculated. The other pig was held in a third room as an uninoculated control.

Virus. For animal infectivity, the Pendik strain of rinderpest virus, passaged twice in cattle at the Plum Island Animal Disease Laboratory, was used. It consisted of a 50% suspension of spleen in blood from infected cattle. At least $10^5$ bovine lethal doses were inoculated into each animal.

Serology. Serum from venous blood collected from individual animals before inoculation and 50 DPI were tested for neutralizing activity in rabbits by the method of Scott and Brown9 and Stone and DeLay.10 The antigen was Nakamura III lapinized virus.

Testing tissues of pigs and peccaries for rinderpest virus. At 50 DPI, the surviving peccary and 2 pigs were necropsied and 20 specimens similar to those used in testing peccary tissues for ASF virus were collected. In the same way described, 10% suspensions of individual specimens were made. The suspensions from 1 animal were pooled and approximately 15 ml was inoculated IM in each of 2 cattle. The cattle were observed for 15 days.

RESULTS

African Swine Fever

Hemadsorption was not observed in peccary leukocyte or bone marrow cultures inoculated with ASF virus though the reaction was produced in inoculated pig leukocyte and bone marrow cultures. No hemadsorption reaction was observed in cultures from the peccaries or pigs from which the erythrocytes were removed and replaced with those from the other species. Inclusion bodies were demonstrated in pig leukocyte cultures but not in those of peccary cells that were stained with Giemsa.

Intramuscular inoculation of 12 peccaries with massive doses of virulent ASF virus did not cause mortality. Pigs, housed with these peccaries, which were given the same dose of virus orally, died 8 DPI. Also, pigs housed in the same room, in contact with the inoculated peccaries and pigs, died 11-18 DPI. Only 1 pig that was exposed by contact with peccaries and pigs which had been inoculated with ASF Dakar virus survived. This pig had a high thermal response and viremia. The pigs that died had clinical signs of acute ASF. Blood samples and spleen suspensions from the pigs taken at the peak of thermal response and at necropsy, respectively, produced hemadsorption in pig leukocyte cultures. In contrast, blood samples obtained from the peccaries at 3, 5, 12, and 25 DPI caused no hemadsorption in pig leukocyte cultures. This negative hemadsorption reaction apparently indicated the
absence of viremia in peccaries.

Tests of serums from peccary blood collected 25, 45, and 60 DPI demonstrated no evidence of precipitating antibodies. Serums obtained at the same intervals from the surviving pig had precipitating antibodies. Precipitation lines were obtained with serum dilutions as great as 1:16.

Inoculation of individual suspensions, prepared from 16 organs, blood, urine, and feces from a peccary killed 21 DPI with ASF, into pig leukocyte cultures did not produce hemadsorption reaction. Pigs individually inoculated with these same suspensions did not develop clinical disease during the 45-day observation period.

Foot-and-Mouth Disease

After 2 days, inoculated peccaries developed thermal response as well as a local reaction at the sites of inoculation. Three DPI, small vesicles appeared at the sites of inoculation on the snouts and nasal mucosa as well as blanching of the skin of the interdigital space of the right front feet. The temperature remained about 102°F until 5 DPI. On the fifth day, numerous vesicles and erosions were on the snouts (Fig. 1), tongues, coronary bands, interdigital spaces, and heels of the right front feet. Secondary lesions developed on the other uninoculated feet. Lesions on the snouts, tongues, and nares healed completely by the tenth DPI while those on the feet remained longer and the skin peeled off, leaving raw and bleeding exposed tissue. Although restricted by soreness, the peccaries were active and continued to eat and drink.

Vesicles appeared on the snouts and feet of uninoculated peccaries 4 days after lesions first developed in the inoculated ones. That the disease in contact animals was more pronounced than that in inoculated ones was evidenced by the higher thermal response and severity of the lesions.

The incubation period in inoculated peccaries was 1 day, and the rise in body temperature continued for 4 to 6 days (Fig. 2). The disease course was about 12 days.

The signs and lesions in pigs were similar to those described for peccaries. However, the thermal response was higher than that recorded for peccaries, and the lesions were more extensive, with large eroded areas on snouts, tongues, and feet.

Tests of 25-DPI serums from the peccaries and pigs indicated neutralizing activity. The VNI of the peccary serums was 3.9, 3.8, 3.9, 5.3, and 4.1 log 10 mouse 50% lethal doses per ml of inoculum. The VNI of the pig serums was 3.8, 3.8, 3.7, and 3.9 (Table 1). All these serums formed precipitation lines when tested by the AGDP technique.

Vesicular Stomatitis

The preinoculation temperature range of the peccaries was 100°F to 101°F. Two DPI, body temperatures increased 1 to 1.5°F (Fig. 4). This rise was accompanied by appearance of small dew-like vesicles at the site of inoculation on the snout and around the sublingual septa. Four DPI, erosions developed on the snout, coronary band, and heels of the right front foot. By 8 DPI, the lesions had healed completely. The disease course was brief and was associated with mild signs and lesions. In the non-inoculated contact peccaries, there was an increase in body temperature. However, small papules developed on the snout, in the mucosa of the
nasal openings, and in the interdigital spaces. The lesions were discernable and localized, but they healed in less than 5 days.

The inoculated pigs had a rise in body temperature to 104-105°F (Fig. 5). Also, extensive large vesicles developed on the snout, its rim, tongue and lower lip, and nasal openings. Blanching and small vesicles developed in the digital space and around the coronary band. Four DPI, a decline in body temperature (103°F) was simultaneous with eruption of the vesicles and appearance of secondary lesions in the other feet. Temperatures dropped gradually and by 11 DPI were 101-102°F. Disease signs appeared in the contact pigs 2-3 days later than in the inoculated pigs, but both signs and lesions were similar to those in the inoculated pigs.

The disease in pigs was more severe than in peccaries. The pigs abstained from eating, probably because the lesions were more extensive and painful than those in the peccaries.

Convalescent serums from peccaries and pigs had detectable neutralizing activity. The VNI log 10/ELD50/ml of the peccary serums from the second trial were 4.6, 3.6, 3.9, 4.6, 4.6, and 3.8 and those of the pig serums were 4.6 and 3.8 (Table 1). Tests for CF activity were negative.

**Vesicular Exanthema of Swine**

Two DPI with VES, type A48 virus, an increase of 1°F in the body temperature occurred in the inoculated peccaries, and there was blanching at the inoculation sites. No vesicles or erosions were noticed in the uninoculated contacts and they appeared clinically healthy and aggressive. In contrast, classical disease signs and lesions appeared in the inoculated and contact pigs.

The second group of peccaries and pigs was exposed to type E54 VES virus. The body temperatures of the 2 inoculated peccaries rose 2°F between the second and third DPI. On the third and fourth day, vesicles appeared on the nares, snouts, and mucosal surfaces of the lower lips. The skin of the interdigital spaces and coronary bands of the right feet first blanched, then formed vesicles. On the fifth DPI, secondary lesions were noticed on the other feet. After 7 days, the uninoculated peccaries also had disease signs. Four days after contact exposure, their temperatures rose 20°F. Ten days later, freshly ruptured vesicles were seen in and around the nares. The next day, more vesicles appeared on the snouts, coronary bands, heels and interdigital spaces. This was followed by extensive erosions and decline in body temperatures. The animals were lame for about 7 days, but all lesions healed and the peccaries recovered in 2 weeks.

Within 1 DPI the pigs' body temperatures (Fig. 4) elevated to 105°F-106°F, and blanching occurred at the inoculation sites. Two DPI, their temperatures were 106°F-107°F, with vesicles covering the entire snout and extending to the upper and lower lips. Also, vesicles on the feet extended to the coronary band. Four DPI, vesicles of the lips and feet ruptured and erosions occurred. The contact-exposed pigs had similar high temperatures and lesions, but these changes were delayed approximately 2 days.

Lameness of the peccaries was less pronounced and lesions were generally smaller and less severe than those of the pigs.

Convalescent serums from the peccaries and pigs had similar high neutralizing antibody titers. The VNI of the 16-DPI serums from peccaries and pigs ranged from
Inoculation into steers of tissue suspensions from the peccary and from 2 pigs did not cause any clinical disease signs during 15 days' observation.

**Hog Cholera**

Five of the 6 inoculated peccaries had temperatures of 101°F to 104°F between 3 and 4 DPI (Fig. 5). During this febrile reaction, rapid respiration, slow movement, and lacrimation were seen. Body temperatures then declined, condition improved, and they rapidly regained strength and aggressiveness. The disease syndrome ran its course approximately 10 DPI.

All pigs had markedly elevated temperatures (105°F-107°F, see Fig. 5) during 2 to 8 DPI. At the onset of disease, a sudden rise in temperature was followed by conjunctival congestion, lacrimation, and accelerated respiration. They then became recumbent and developed diarrhea. Six to 8 DPI, they lay on one side and became comatose. They were killed *in extremis* 9 DPI. At necropsy, all pigs had typical HC lesions including epicardial hemorrhage, intestinal congestion and hemorrhage, petechiation of the kidneys, ulceration at the illeocecal orifice and swollen lymph nodes.

**Rinderpest**

All 6 peccaries had a severe febrile reaction 3 to 4 DPI with rinderpest virus (Table 2). By 4 DPI, the peccaries huddled, had hunched backs, and developed profuse diarrhea which later was tinged with blood. Beginning 6 DPI, temperatures began to gradually decline, their coats lost luster, they lay on their briskets, and loss of weight was evident. Two days before death, an intermittent cough was heard and a watery-sanguinous diarrhea developed. At 6, 9, and 10 DPI each, 1 peccary died and at 8 DPI, 2 others died.

At necropsy, the blood was thick and dark red, the skeletal and mesenteric lymph glands were swollen and edematous and the lungs were severely congested and studded with penumonic areas. The heart was congested with petechiation along the coronary artery. The heart blood was not coagulated and, in most cases, the pericardial sac contained an excess of serous fluid. The trachea was full of blood-tinted, frothy fluid, Mucosae of the epiglottis, larynx and trachea were congested with many scattered petechial hemorrhages. Erosions of the epithelia of the lower lip mucosa and adjacent labial gum surfaces were numerous.

The fundic area of the stomach was congested and edematous, and the intestines relatively empty. Peyers patches were clearly visible from the serosal surfaces of the intestines; they were edematous, congested and raised but not hemorrhagic. Their mucosal surfaces were covered with pseudo-membranes.

In the large intestines, the lesions varied from severe congestion to petechiation and ecchymosis. In areas, the mucosa was covered with thick mucus. The most significant lesion was on the terminal 10 inches of the mucosal surface of the large colon where severe hemorrhage and ecchymosis and, in some cases, erosions with free bleeding were evident. No macroscopic changes were observed in the livers, kidneys or spleens.

One of 2 inoculated pigs had a slight thermal response between 3 and 8 DPI. The temperature range was 103.4 to 103.8°F. The 2 pigs appeared clinically
healthy during a 50-day observation period.

Marked serum neutralizing activity was found in the 50-DPI serum of the peccary which survived. Similar levels of neutralizing activity were encountered in serums obtained at the same time from the inoculated pigs. The VNI of the peccary serum was 4.5 and those of the pigs were 4.5 and 5.0 Log 10 rabbit 50% lethal doses per ml (Table 1).

Rinderpest virus was not detected in tissues collected 50 DPI from an inoculated peccary and 2 inoculated pigs, indicating that these animals were not carriers.

DISCUSSION

American white-collared peccaries were resistant to infection with 3 ASF viruses. The design of these experiments assured intensive exposure to ASF virus, vis. 1) massive doses of ASF virus were inoculated IM; 2) they were placed in direct contact with ASF-inoculated pigs which subsequently died of the disease; and 3) they were exposed to ASF-contaminated premises. Inability to show evidence of ASF virus infection in cultures of leukocyte and bone marrow cells from peccaries was another indication of resistance to the disease. Further evidence to substantiate this observation is the failure to detect viremia at frequent intervals following inoculation. In addition, precipitating antibodies were not demonstrated by the AGDP test.

Foot-and-mouth disease, VS, and VES lesions were produced in peccaries by intradermal inoculation of the snouts and tongues, the mucosa of the mouth, and skin in the interdigital spaces. Contact-exposed peccaries developed similar thermal responses and vesicles. The signs and lesions of the diseases in peccaries were generally similar to those developed in pigs, but were milder and of shorter duration. The 2 species were equally susceptible to FMD virus, but there may be a difference in their susceptibility to different VES virus types. An inapparent form of disease resulting from exposure to VES type A48 virus contrasts with an overt form caused by type E54 virus. Exposure of the peccaries and pigs to the 3 vesicular diseases elicited similar, marked neutralizing activity. Foot-and-mouth disease and VS convalescent serums were negative for CF antibodies.

Acute HC signs developed in inoculated pigs, but a milder and shorter disease course was experienced by similarly treated peccaries.

The comparative reactions of the peccaries and pigs to rinderpest virus were of interest. Peccaries suffered from an acute febrile disease and 5 of 6 inoculated, died. Although a peccary survived, it underwent acute disease. Postmortem lesions were characterized by involvement of the lymphatic tissues and the epithelial mucosa of the intestinal tract. The inoculated pigs experienced a mild transitory thermal response, indicating an inapparent disease form. The European pig, unlike Asian pigs, is resistant to rinderpest infection. However, an acute disease in peccaries was similar to that of the Asian pig reported by Molinier.

Finally, the information developed indicates clearly the susceptibility of peccaries to FMD, VS, VES, and rinderpest viruses. The role these animals may play in disseminating these diseases should not be disregarded.
SUMMARY

American white-collared peccaries and pigs contracted foot-and-mouth disease, vesicular stomatitis, vesicular exanthema of swine, hog cholera, and rinderpest by inoculation or direct contact. The clinical signs and courses, except for those of rinderpest, were milder and of shorter duration in peccaries than in pigs. In both species, exposure to the 3 vesicular diseases resulted in marked neutralizing activity in convalescent serums. Inoculation of rinderpest virus into peccaries induced a severe acute disease with death and postmortem lesions similar to those in cattle and Asian pigs. Pigs were resistant to the same rinderpest virus, exhibiting only a slight transitory thermal reaction. Inoculation with 3 African swine fever viruses failed to infect peccaries. The peccaries remained clinically healthy, did not develop viremia or precipitating antibodies, and were not virus carriers at 21 DPI. The hemadsorption reaction could not be induced by inoculation of African swine fever virus into peccary leukocytes or bone marrow cultures.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Joan Grohoski and Mr. P. Mikiciuk for their technical assistance.
### TABLE 1.

**VIRUS NEUTRALIZATION INDICES OF SERUMS FROM SWINE AND PECCARIES CONVALESCENT FROM FOOT-AND-MOUTH DISEASE, VESICULAR EXANTHEMA, AND RINDERPEST**

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Peccaries</th>
<th>Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot-and-mouth disease</td>
<td>3.9, 3.8, 3.9, 5.3, 4.1</td>
<td>3.8, 3.8, 3.7, 3.9</td>
</tr>
<tr>
<td>Vesicular stomatitis</td>
<td>3.6, 3.8, 3.9, 4.6, 4.6, 4.6</td>
<td>3.8, 4.6</td>
</tr>
<tr>
<td>Vesicular exanthema of swine</td>
<td>4.3, 5.3, 5.3, 5.3, 6.3</td>
<td>6.3, 6.3</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>4.5</td>
<td>4.5, 5.0</td>
</tr>
</tbody>
</table>

VNI = Virus neutralization index

### TABLE 2.

**THERMAL RESPONSE AND MORTALITY OF PECCARIES AND PIGS FOLLOWING INFECTION WITH RINDERPEST VIRUS**

<table>
<thead>
<tr>
<th>DPI</th>
<th>Peccaries</th>
<th>Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>101.6</td>
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</tr>
<tr>
<td>3</td>
<td>102.6</td>
<td>100.6</td>
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<tr>
<td>4</td>
<td>104.0</td>
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</tr>
<tr>
<td>5</td>
<td>103.0</td>
<td>102.4</td>
</tr>
<tr>
<td>6</td>
<td>103.0</td>
<td>101.0</td>
</tr>
<tr>
<td>7</td>
<td>102.0</td>
<td>100.8</td>
</tr>
<tr>
<td>8</td>
<td>101.1</td>
<td>Died</td>
</tr>
<tr>
<td>9</td>
<td>100.0</td>
<td>Died</td>
</tr>
<tr>
<td>10</td>
<td>100.6</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>100.4</td>
<td>Died</td>
</tr>
<tr>
<td>12</td>
<td>100.1</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>100.2</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

*Uninoculated control.*
Fig. 1. Erosion on a peccary's snout after inoculation with a foot-and-mouth disease virus.
Fig. 2. Thermal response of peccaries and pigs to foot-and-mouth disease O₁ (CANEFA 2) virus.
Fig. 3. Thermal response of peccaries and pigs to vesicular stomatitis viruses.
Fig. 4. Thermal response of peccaries and pigs to vesicular exanthema of swine viruses.
Fig. 5. Thermal response of peccaries and pigs to virulent hog cholera virus.
BIBLIOGRAPHY

VESICULAR DISEASE COMMITTEE
1969 Report

Chairman: E. C. Sharman, Hyattsville, Maryland

The vesicular disease committee reports as published in the proceedings of the United States Animal Health Association provide a continuing summary on the status of vesicular diseases in the United States and in other parts of the world and on vesicular disease research.

INCIDENCE OF VESICULAR DISEASES

Foot-and-Mouth Disease – The incidence of foot-and-mouth disease (FMD) in 1968 and 1969 has continued relatively low in most European countries. FMD virus types O and C were the predominant types reported during the last part of 1968 and the first half of 1969. Type A was also reported in Czechoslovakia, Denmark, and Italy. Ireland, Iceland, Norway, Sweden, Finland, Netherlands, Luxembourg, Austria, Bulgaria, Albania, Cyprus, and Malta did not report outbreaks during 1968 or the first half of 1969. Poland remained free during 1968 but experienced a small outbreak in early 1969. Belgium and Denmark reported outbreaks in 1968 but have reported none in the first half of 1969.

Several countries which reported no outbreaks in 1967 experienced outbreaks in late 1968 and 1969. Romania, Czechoslovakia, and Yugoslavia, which had not reported outbreaks of FMD since 1966, experienced outbreaks in 1968.

Yugoslavia experienced an outbreak with type O in pigs and sheep in April and May of 1968. The outbreak was brought under control by slaughtering all exposed susceptible animals and vaccinating cattle within a 20 km radius of the outbreak with a monovalent type O vaccine. An outbreak of type C virus involving Romania, Czechoslovakia, Yugoslavia and Hungary occurred in October 1968. Yugoslavia and Hungary successfully suppressed the outbreak. Romania reported an outbreak in January 1969. Czechoslovakia continued to experience virus type A and O outbreaks during 1969.

France reported 22 outbreaks in 1967, 40 in 1968 and 33 through August 15, 1969, resulting from types O and C virus. France vaccinates more than 18 million cattle annually.

Italy reported 20 outbreaks of FMD in 1968 and 123 outbreaks during the first seven months of 1969. Most of the outbreaks were caused by type C virus although types O and A were also isolated. Spain reported 561 outbreaks during 1968 and 328 through the first six months of 1969.

Portugal reported 923 outbreaks in 1968 and 155 outbreaks through June 1969.

The incidence in West Germany decreased from 3,332 outbreaks in 1967 to 68 in 1968 and 12 outbreaks through April 15, 1969. All of the outbreaks in 1968 were caused by type O virus. Annual vaccination with trivalent vaccine is compulsory. Young animals are vaccinated twice during their first year of life.
The Middle East experienced a serious outbreak of foot-and-mouth disease beginning in May 1969. Lebanon reported 47 outbreaks in the month of May. Syria reported 104 outbreaks through July with most occurring since May. The disease was first reported in sheep and goats in Lebanon and became widespread in Lebanon and Syria.

Outbreaks of FMD have remained high in certain countries. India reported over 15,000 outbreaks in 1968, up from 2,261 in 1967. Iraq experienced more outbreaks in the first 6 months of 1969 than during all of 1968.

In the continent of Africa, Algeria reported 833 outbreaks in 1967, 4 outbreaks in 1968 and none during the first half of 1969. Libya reported a large number of outbreaks in 1967 and dropped to only 91 cases in 1968. Virus types identified in Africa during 1968 and 1969 were Angola – types A, O and SAT 2, Botswana – SAT 1, Egypt – type A, Ghana – type A, SAT 1 and SAT 2, Kenya – types A and O, Libya – type O, Mozambique – SAT 1, Nigeria – type A and SAT 1, South Africa – SAT 1 and SAT 2, South West Africa – SAT 2, Tanzania – SAT 2, Tunisia – type C, Uganda – type A, O and SAT 2, and Upper Volta – SAT 2. The World Reference Laboratory for Foot-and-Mouth Disease in Pirbright, England, reported SAT 1 in Ghana and SAT 2 in Namibia. This was the first report of these types in Ghana and Namibia.

Foot-and-Mouth disease in South America is enzootic. Chile experienced a severe epizootic in 1968 cause by virus subtype A26. The Regional Program in the Province of Llanquihue continues while measures are being finalized to extend the campaign to a national level, with the financial support of the Inter-American Development Bank.

Foot-and-Mouth disease types A and O appeared throughout Ecuador during 1968 and the first half of 1969. Outbreaks in Argentina declined from 4,634 in 1967 to 1,417 in 1968. There were 921 outbreaks reported in 1969 prior to August. In addition to vaccination of cattle, compulsory vaccination of sheep over 3 months of age was started in some areas of Argentina in 1968. Brazil experienced an increase in outbreaks from 116 in 1967 to 268 in 1968 and has reported more outbreaks in the first 5 months of 1969 than all of 1968.

Foot-and-Mouth disease has never been diagnosed in Central American or Panama. However, the continuing threat of FMD gaining entry from South America will be greatly enhanced upon completion of the Pan American Highway through the Darien Area.

In June 1968 the Animal Health Division, in response to a request from the International Regional Organization for Plant and Animal Health assigned two veterinarians to Central America to assist the local governments in establishing a vesicular disease surveillance program and developing an eradication plan for use if FMD occurs in the area.

The 1967-1968 outbreak of foot-and-mouth disease (FMD) in Great Britain ended with a case found on June 4, 1968. In the worse recorded epizootic of FMD for Great Britain 433,987 animals were slaughtered on 2,346 farms. Great Britain’s Minister of Agriculture, Fisheries, and Food appointed a Committee of Inquiry on Foot-and-Mouth Disease “to review the policy and arrangements for dealing with foot-and-mouth disease in Great Britain and to make recommendations”. The Report of this Committee will be presented in two parts. Part one
suggests ways by which the risk of introduction of FMD virus into Great Britain, and of future epidemics might be reduced. Reproduced below is the Recommended Policy from Chapter VIII of the report:

**Recommended Policy**

"216. Our recommendations for reducing the risks of the introduction of foot-and-mouth disease, and for controlling its spread, are based on a consideration of the various policies including slaughter, import control and vaccination described in Chapter VII, and on our judgment as to which of these are appropriate to Great Britain."

"217. We have shown in earlier chapters that there are substantial gaps in our scientific knowledge of foot-and-mouth disease, particularly in relation to some aspects of epidemiology. It is not possible therefore to make recommendations regarding prevention and control policies purely on scientific grounds."

"218. We have attempted to assess the gradient of risks of the introduction of foot-and-mouth disease in meat and meat products. The degrees of risk will depend on Government policy. The risks would be greatest if there were unlimited importation of meat and meat products from all countries irrespective of their foot-and-mouth disease status, and least if meat were imported only from foot-and-mouth disease-free countries such as Australia and New Zealand. Between these extremes there are varying degrees of risk depending on what restrictions are applied to imports of meat and meat products and offal, and on whether such imports have their origin in countries where the disease is sporadic and the risks are less, or in countries where the disease is endemic and the risks are greater. We have pointed out that the major risks derive from the persistence of foot-and-mouth disease virus in bones, offal and lymph glands, and we think that if a policy is adopted which excludes these dangerous components, the reduction of risk would be almost equivalent to that which would be achieved by a complete ban on meat imports."

"219. Slaughter, which is the best method of eradicating the disease, is essential whatever policy is adopted, but the adoption of a policy which relies on the slaughter policy alone should, in our view, be dependent either on a complete ban on imports, or at least on the exclusion of the dangerous components of meat, from countries or areas of countries where foot-and-mouth disease is endemic. If these dangerous components are not excluded we think it essential that some form of vaccination should be introduced."

"220. General vaccination gives a large measure of protection but this advantage has to be balanced against the disadvantages attaching to the disturbance of normal farming practice, the diversion of veterinary manpower and the very considerable cost (see paragraphs 177 to 195)."

"221. Ring vaccination would not limit the number of primary outbreaks but it could limit spread. Although it has considerable disadvantages (see paragraphs 200 to 205), we think that in certain conditions ring vaccination could be a useful adjunct to slaughter."

"222. We recommend that the slaughter policy, which we consider to be the best method of eradicating foot-and-mouth disease when it occurs in Great
Britain, should be continued. This policy by itself should only be adopted if the conditions of meat import policy are such as to reduce substantially the risks of primary outbreaks occurring. If such conditions of meat import policy are not put in force we would recommend that the slaughter policy should be reinforced by a ring vaccination scheme (see paragraphs 173 to 175 and 196 to 206).

II. The conditions of meat import policy which in our view are necessary to enable the slaughter policy by itself to be continued and which we recommend are:

(a) The ban on imports of mutton, lamb and pigmeat from countries or areas of countries where foot-and-mouth disease is endemic should continue (see paragraphs 150 and 152).

(ii) Imports of mutton and lamb offal and pig offal from countries or from areas of countries where foot-and-mouth disease is endemic should be limited to offal processed in such a manner as to destroy foot-and-mouth virus (see paragraphs 14 to 17, 144, 164 and 165).

(b) Because there is a high risk of introducing foot-and-mouth disease into Great Britain by importing carcase beef and beef offal from countries or from areas of countries where foot-and-mouth disease is endemic, on strictly animal health grounds there should be a complete ban on all such imports (see paragraphs 14 to 17 and 144 to 170).

(c) Alternatively if for social, political or commercial reasons the recommendation in (b) is not accepted, imports of carcase beef and beef offal from countries or from areas of countries where foot-and-mouth disease is endemic should be limited to—

(i) boned-out beef (see paragraphs 14 to 17, 165 and 166); and

(ii) beef offal processed in such a manner as to destroy foot-and-mouth disease virus (see paragraphs 14 to 17 and 164 to 165).

III. We recommend that:

(a) Our veterinary staff in South American countries should be strengthened in order that standards of public health inspection acceptable to the British Government can be ensured, and so render the retention of lymph glands in boned-out cuts of meat unnecessary, and in order to assist the implementation of the Bledisloe arrangements (see paragraph 168).

(b) Because some countries in which foot-and-mouth disease is endemic have well-defined areas which for geographical or other reasons are free of the disease and which could be accepted as safe sources for imports of meat, provision should be made to permit imports of meat, under suitable safeguards, from such areas (see paragraph 49).

IV. We recommend that contingency plans for the application of ring vaccination should be kept in constant readiness. They could be put into operation should our recommendations in II not be successful in limiting the number of outbreaks (see paragraph 196 to 206 and 221).

V. We recommend that the importation of meat and meat products from all sources be subject to revocable conditional licenses (see paragraph 146).

VI. We recommend that adequate facilities for cleansing and disinfection of
VESICULAR DISEASE

vehicles and persons engaged in the transport of livestock should be a legal requirement at appropriate points of entry into Great Britain (see paragraph 141).

V. We recommend an expansion of research work on foot-and-mouth disease, particularly in epidemiology, on the lines suggested in paragraphs 213 to 214, and the use of epidemiological teams in the field (see paragraph 198 and 215)."

VESICULAR STOMATITIS

United States — There were seventy vesicular disease investigations during calendar year 1968 but no virus was isolated. Serums from 5 premises and tissue from one, collected in Louisiana, were serologically positive for New Jersey type vesicular stomatitis (VS) on the complement-fixation (CF) test. Similar reactions were found with single serum samples submitted from Georgia, Arkansas and Mississippi. During the first nine months of 1969 there were 27 negative vesicular disease investigations.

Epidemiology — Bovine serums from six states and Puerto Rico were surveyed by serum neutralization (SN) test for antibodies against VS virus. This report summarizes the results obtained with 1042 serums received between Fall 1968 and July 1969.

TABLE 1

<table>
<thead>
<tr>
<th>State</th>
<th>Number of Samples Received</th>
<th>Last Diagnosed VS in State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>220</td>
<td>1964</td>
</tr>
<tr>
<td>Georgia</td>
<td>180</td>
<td>1964</td>
</tr>
<tr>
<td>Indiana</td>
<td>200</td>
<td>1925</td>
</tr>
<tr>
<td>Louisiana</td>
<td>62</td>
<td>1968</td>
</tr>
<tr>
<td>New Jersey</td>
<td>80</td>
<td>1953</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>200</td>
<td>1966</td>
</tr>
<tr>
<td>Texas</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Total Serums Received and Tested 1042

Except for those from Louisiana, serums were selected from animals born since the last known occurrence of VS in each state. Serums were selected from those submitted for the brucellosis test and were chosen to include counties with a history of VS. Samples were frozen in dry ice and submitted to the National Animal Disease Laboratory for testing.

All serums were negative for Indiana VS antibody by the neutralization test. Serums from Indiana, New Jersey and Puerto Rico did not neutralize New Jersey
VS virus. Two serums from Texas demonstrated insignificant amounts of neutralizing antibodies as did one of 220 received from Alabama.

Five of 180 Georgia serums tested reacted with NJ virus on the SN test. One was classified suspicious. Four serums with significant titers were from cattle in Northwest Georgia, and were from animals listed as four years of age. These samples were collected during December 1968 and January 1969. Some of these animals probably were present when New Jersey VS was in the area during 1964.

Of 62 serums received from Louisiana, five (two and three year old animals) revealed significant antibody titers to New Jersey VS virus. Three positive serums were from one herd in Sabine Parish where VS was last reported in 1960. None of eight serums which neutralized VS virus had complement-fixing antibodies to VS virus.

The survey for vesicular stomatitis in wildlife continued during the period of this report with the collection and neutralization testing of serums from the following species in Louisiana, Texas, and Colorado:

<table>
<thead>
<tr>
<th>Rodent Survey</th>
<th>Total No.</th>
<th>New Jersey VS</th>
<th>Indiana VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louisiana</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline Wildlife Management Area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LaSalle Parish, La.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peromyscus sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmodon hispidus</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>juvenile (Cotton Rat)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Texas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mission, Hidalgo Co.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collected October 1969</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigmodon hispidus</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peromyscus leucopus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(White-footed mouse)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liomys irroratus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mexican pocket mouse)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rio Grande City, Starr Co.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Collected January 1969</td>
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<td></td>
<td></td>
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<tr>
<td>Sigmodon hispidus</td>
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<tr>
<td>Neotoma micropus</td>
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<tr>
<td>(Southern plains woodrat)</td>
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</tr>
<tr>
<td>Onychomys leucogaster</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(Northern grasshopper mouse)</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Peromyscus sp.</td>
<td>3</td>
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</table>

<table>
<thead>
<tr>
<th>Deer Survey</th>
<th>Total No.</th>
<th>New Jersey VS</th>
<th>Indiana VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louisiana</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louisiana Wildlife and Fisheries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pass-A-Loutre Plaquemines Parish</td>
<td>117</td>
<td>IP, 3S*</td>
<td>0</td>
</tr>
<tr>
<td>Louisiana</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Antelope Survey Total No. New-Jersey VS Indiana VS
Colorado
Various hunting areas of the state 96 1** 0

* Three were suspicious with titers of 1:8 and one weakly positive at 1:16.
One-hundred-thirty-eight deer samples from the southeastern states were received in August and have not yet been tested.

** New Jersey VS serum neutralization titer of 1:32.

The above studies were made possible by the assistance of Animal Health Division, State Brucellosis laboratories and State wildlife personnel in the cooperating states.

Sentinel Mice

Forty-seven litters of one-day old suckling mice were exposed suspended under Causey hoods in the Saline Game Management Area of LaSalle Parish, Louisiana, between May 10 and June 6, 1969. Sick and dead mice were collected from eight litters during the 14 day holding period. No virus was isolated from brains of these mice.

Field Trials in Georgia designed to determine presence of VS virus have continued during 1968. Blood samples were collected in 1966, 1967, and 1968 from 7 herds located in Carroll and Heard Counties. None of the samples collected in 1968 from 2 and 3 year old animals had neutralizing antibodies. Serum from three of the 4 year old animals had neutralizing activity for New Jersey VS virus. Animals 4 years of age and older may have been on the farms during the 1964 outbreak. The following is a summary of the 1968 tests:

<table>
<thead>
<tr>
<th>Herd</th>
<th>2 yr.</th>
<th>3 yr.</th>
<th>4 yr.</th>
<th>5 yr.</th>
<th>6 yr.</th>
<th>7 yr.</th>
<th>8 yr.</th>
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<tbody>
<tr>
<td>1</td>
<td>*</td>
<td>*</td>
<td>0/1</td>
<td>2/8</td>
<td>6/21</td>
<td>3/8</td>
<td>9/18</td>
</tr>
<tr>
<td>2</td>
<td>0/4</td>
<td>0/5</td>
<td>0/2</td>
<td>4/14</td>
<td>4/9</td>
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<td>0/4</td>
<td>2/10</td>
<td>5/21</td>
<td>5/10</td>
<td>8/12</td>
</tr>
<tr>
<td>6</td>
<td>*</td>
<td>0/1</td>
<td>0/18</td>
<td>1/24</td>
<td>9/33</td>
<td>15/41</td>
<td>10/20</td>
</tr>
<tr>
<td>7</td>
<td>0/3</td>
<td>3/14</td>
<td>10/21</td>
<td>7/12</td>
<td>1/2</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0/5</td>
<td>0/13</td>
<td>3/57</td>
<td>19/95</td>
<td>33/111</td>
<td>28/80</td>
<td>32/63</td>
</tr>
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</table>

*None tested in this age group.

Mexico – During calendar year 1968 veterinarians of the Joint Mexico – U.S. Commission for the Prevention of Foot-and-Mouth Disease carried out 68 field and laboratory investigations for vesicular diseases in Mexico. Seventeen proved to be New Jersey VS, 14 were Indiana VS, and 20 fixed complement with both types.
Serums collected from 16 herds fixed complement for both New Jersey and Indiana virus. Additional serums collected from 4 of these herds fixed complement for New Jersey virus only, while additional serums from 12 of these herds fixed complement for Indiana VS virus only. Separate serum samples from another herd fixed complement for New Jersey VS virus and Indiana VS virus.

In 1968, as in 1967, most of the cases occurred in the southern half of Mexico. Infected premises nearest the United States were in north central Chihuahua and northeast Coahuila.

From January 1 through October 8, 1969, 47 investigations were made. Of the 19 specimens which were positive for VS, 8 reacted with New Jersey VS, 6 with Indiana VS, and 5 with both.

RESEARCH ON VESICULAR DISEASES

**VESICULAR EXANTHEMA OF SWINE** — [Although] vesicular exanthema of swine (VES) is extinct in all parts of the world, some research is still done on the virus. The virus was chemically and physically characterized by Wawrzkiewiez et al. Its sedimentation coefficient was 160 to 170 S in sucrose, its density approximately 1.37 g/ml in cesium chloride and it had a single-stranded 35 to 38 S RNA core. Its diameter of 35 to 40 μ and appearance in the electron microscope were similar to feline picorna virus.

**VESICULAR STOMATITIS** — Studies to determine the role of insects in the transmission of vesicular stomatitis virus (VSV) have increased. Of particular interest is the work of Bergold et al on mosquito transmission. They found that VSV multiplied to 4.5 logs of infectivity in 7 days in intrathoracic-inoculated *Aedes aegypti*. The concentration of VSV was highest at 2 to 3 days postinoculation. The highest concentration was found in the thoracic and abdominal ganglion during early stages of infection and the salivary glands after 20 days. The Indiana but not New Jersey strain was easily expelled from the gut lumen by slight pressure leading the authors to conclude that virus might be shed during oviposition. Electron microscopy confirmed the presence of virus particles in the salivary glands as well as other organs. Infected mosquitoes transmitted infection to baby mice from 2 to at least 20 days postinoculation. However, a difference in the transmission rate was found between Indiana and New Jersey VSV: 45% of mosquito lots were able to transmit the Indiana strain to 11% of exposed baby mice, whereas only 20% of New Jersey strain infected lots were able to transmit to 5.2% of exposed mice.

Two cell lines which originated from *Aedes* mosquito tissues were found susceptible to infection with both Indiana and New Jersey strains of VSV. However, neither virus caused cytopathogenic effect (CPE). It was of interest that the other arboviruses that also grew in these cell lines were all mosquite-borne viruses. Tick-borne viruses did not grow in these cells. This worker suggests the use of these cell lines to screen for viral agents that might be mosquito-borne.

Printz has described some properties of VSV adapted to the *Drosophila* fly. He found it reduced the longevity of the fly as well as the female fertility. This fly-adapted strain grew better in tissue culture at 27°C than at 37°C and its infectious titer in flies exceeded culture titers by 10-fold.

Several workers have reported on the growth of VSV and interference of its
growth by chemical or physical agents. Crick et al\textsuperscript{5} found virus particles of two different growth rates in tissue cultures inoculated with crude material. The slower-growing virus produced large amounts of a non-replicating particle that caused auto-interference in VSV growth. This slower-growing virus was favored when high multiplicity inoculums were used.

Rifampicin\textsuperscript{6}, Mitomycin C, Actinomycin D and ultraviolet light\textsuperscript{7} have all been shown to have an effect on the growth of VSV in tissue cultures. The effects noted were a function of the dose used and the phase of the replicative cycle. Heine and Galasso\textsuperscript{8} also found the infectivity of VSV reduced and the particle distorted if the virus was suspended in aqueous solutions which had previously been exposed to x-rays.

Arstila et al\textsuperscript{9} have described the requirements for demonstrating hemagglutination with VSV grown in baby hamster kidney (BHK) cells.

Wagner et al\textsuperscript{10,11} have reported rather extensive work on the capsid protein structure and chemical composition of VSV. Cartwright et al\textsuperscript{12} found that the spike-like projections on the VSV particle were responsible for cell adsorption as well as stimulation of neutralizing antibody. Their destruction by trypsin digestion caused lowered infectivity and loss of complement-fixing activity.

FOOT-AND-MOUTH DISEASE – The severe outbreak of foot-and-mouth disease (FMD) in Great Britain in 1967-68 and the resultant committee of inquiry under the chairmanship of the Duke of Northumberland\textsuperscript{13} have had an effect on the direction of FMD research. The very rapid spread and appearance of numerous foci almost simultaneously has led many to believe that spread of virus by the wind may play a significant role in the epizootiology of FMD. This problem has been approached from two aspects – laboratory studies on air-borne transmission and study of the disease pattern during outbreaks and its correlation with meteorological conditions at that time.

Sellers and Parker\textsuperscript{14} by using large volume sampling methods found pigs excreted more virus into the air than cattle or sheep. Pigs shed an average of $10^6$ ID\textsubscript{50} of infectious virus over a 5-day period and cattle and sheep about $3 \times 10^4$ ID\textsubscript{50} over the same period. Maximum virus was shed from cattle and pigs at 41 hours after infection when lesions had generalized but from sheep maximum shedding occurred at 17 hours after inoculation and before lesions were observed. The authors suggest that the virus excreted as aerosol is produced in the upper respiratory tract. They theorize that with relative humidity greater than 70\% and low temperature the virus could survive up to 100 km as an airborne entity.

Smith and Hugh-Jones\textsuperscript{15} studied the meteorological conditions that prevailed during 4 unrelated British outbreaks. Since they found no evidence to the contrary, they concluded that spread of FMD by weather elements is theoretically possible. Wind direction and speed, temperature and relative humidity were the factors considered.

A detailed epidemiological study of the outbreak in Worcestershire which led to the investigations by the Northumberland Committee was done by Henderson.\textsuperscript{16} He concluded that virus from a primary focus on each of three pig farms could have been carried by the wind and could have caused 26 additional foci in the area. He found that housing cattle in sheds offered no protection but delayed the onset of disease by one day over cattle kept in the open.
The virus of FMD was found to survive on the body of the house fly up to 72 hours and within the fly up to 48 hours. The infectivity was studied by inoculation of washings from the flies into guinea pigs.

The role of the carrier animal in the epizootiology of FMD is still unknown. The Northumberland report concluded after expert testimony that “From the material that has been collected and surveyed it would seem that only in exceptional cases are recovered animals able to transmit foot-and-mouth disease and, therefore, would seem to play a very small role in the epizootiology of the disease”. The carrier state continues to be studied in hopes of clarifying its role. McVicar and Sutmoller have shown that a very high percentage of sheep and goats become virus carriers after FMD infection either experimentally induced or from contact with infected cattle.

Of interest is the simultaneous isolation of carrier viruses of two different types during routine testing of Brazilian cattle for shipment to Venezuela at the Pan American Foot-and-Mouth Disease Center. One of the viruses, type C, is not present in Venezuela.

Potential transmission of FMD by other means continues to be studied. FMD virus growth with formation of microscopic lesions has been shown in the skin of cattle by Gailiunas. Forbes and Cottral studied the simulated conditions of commercial drying of blood on FMD virus survival and found that there was little likelihood of virus survival under such treatment.

Hedger et al. found that FMD virus could be isolated from the pharynx of wild African buffalo. They suggest that wild game animals may be an important reservoir for infection of domestic stock in Africa. Of interest was the observations that the virus they isolated had not been present in the area for 18 months.

The effect of FMD on the individual animal continues to be studied in order to expand our knowledge of the symptoms in the several susceptible species. It is increasingly evident that FMD in sheep may not be the spectacular syndrome seen in cattle and swine. Research workers in Uruguay concluded that infected sheep would most likely not be noticed in the field.

FMD infection has been found to have a serious effect on semen quality and breeding capabilities of infected bulls. In one study, return of semen quality occurred after recovery of the animals from infection. Others, however, found permanent damage to the breeding capacity. Most of this work has been done in Russia where FMD outbreaks in assemblies of bulls used for artificial insemination seem to be a problem.

Potel has studied, in considerable detail, the nature of the heart lesions of FMD virus infection. He concluded that a great deal of the damage and failure of the heart was caused by white cell infiltration in response to myocardial destruction.

A very interesting report on an FMD-like disease has been made by Italian and British workers. A disease appeared in two pig farms in Italy which had recently received pigs for fattening. The disease was initially diagnosed as FMD but on several tests it failed to serologically type in both Italy and England. It could be transmitted in pigs by inoculation of infected epithelium or culture fluids. Further study of the infectious agent including stability at pH 5.0 led these workers to classify it as an enterovirus.
Vaccination against FMD is still of major importance in most parts of the world. In Latin America, several loans of considerable value for vaccination campaigns are under consideration by the Inter American Development Bank. Loans have already been granted to Chile, Argentina and Uruguay. One of the basis of the loans is a regional consideration of control campaigns rather than individual countries. All have a clause requiring adequate technical support of the participating agency. While there is no doubt that current vaccines when properly applied have a considerable impact on FMD incidence in countries where the disease in enzootic, continuing research will be required to advance vaccines to higher efficiency levels. Experiments have been done and are continuing, to study the potential of concentration and purification of viral antigens in vaccines.28,29,30

There have been reports of some adverse reactions in cattle after use of vaccines of various types. In Germany, there is rather clear evidence that vaccine prepared from virus grown in BHK cells causes significantly more reaction than Frenkel-produced virus. The reactions take the form of immediate and delayed hypersensitivity. Reacting cattle remain sensitive to subsequent vaccination. The nature of the reactions still requires considerable research for clarification. Mayr and Bibrack31 have reported on allergic reactions in animals from antibiotics. All FMD vaccines contain antibiotics of various types for control of saprophytic bacteria and molds during production. Wittmann and Bauer32 have found adverse reactions in pigs from vaccine containing modified Freund's adjuvant. Abscesses were found in a large percentage of experimentally vaccinated pigs. The reactions were not associated with vaccine dose or site of inoculation.

The problem of hypersensitive vaccine reaction is being studied in several laboratories. The several vaccine components are being researched for the possible cause of the reactions. Purified virus offers a solution if it should develop that BHK FMD vaccine produced virus is the cause. Other possible causes include the inactivant and its effect on other components and the adjuvant.30

Recently concluded cooperative experiments between the Plum Island Animal Disease Laboratory and the Pan American Foot-and-Mouth Disease Center showed a high degree of protection could be expected in sheep with currently available vaccines. Because of these findings, the Center now recommends routine vaccination of sheep in those countries with vaccination campaigns.

The virus of FMD is still studied from many basic aspects. The chemistry of the surface structure has been studied in considerable detail.33,34 The protein antigenic properties in relation to antibody specificity have been reported by Cowan35 and Rowlands et al.36 The molecular biology of FMD virus replication in relation to RNA and polyribosome has been studied by Ascione and Arlinghaus.37 Richmond38 found that swine leukocyte cultures treated with phytohemagglutinin produced an interferon-like substance that markedly reduced FMD virus growth in cells treated with cell-free extract of the substance. As well as reduction in titer, plaque size was greatly reduced. Synthetically-produced chemical substances simulating double-stranded RNA are also being studied for their interferon-inducing capacities.

**RECOMMENDATIONS**

The Vesicular Disease Committee has examined the report of the Committee of
Inquiry on foot-and-mouth disease which has recently been published by the British Ministry of Agriculture. The British Committee considered ways by which the risk of the introduction of foot-and-mouth disease into Great Britain might be reduced and examined policies and procedures to be followed in dealing with the disease should it occur again in that country.

The Vesicular Disease Committee of this association recommends that the Secretary of the U.S. Department of Agriculture be contacted with the suggestion that a similar in-depth study be made in the United States. The committee further recommends that the study group include major interest groups, related to livestock production and marketing. The study group should address themselves to all aspects of the problem to include, but not be limited to risks of the disease being introduced into the United States, methods of controlling and eradicating the disease should it occur, and additional research required. The study group should begin deliberations on this subject immediately.
REFERENCES


AMERICAN ASSOCIATION OF
VETERINARY LABORATORY DIAGNOSTICIANS

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Dr. E. P. Pope ....... Secretary-Treasurer

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Secretary-Treasurer
CONSTITUTION AND BY-LAWS

of the

AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

CONSTITUTION

ARTICLE I – NAME

The name of the Association shall be the American Association of Veterinary Laboratory Diagnosticians.

ARTICLE II – UNITED STATES ANIMAL HEALTH ASSOCIATION AFFILIATION

The American Association of Veterinary Laboratory Diagnosticians shall be an affiliate of the United States Animal Health Association in accordance with a memorandum of agreement mutually agreed upon between the two parties and approved by a two-thirds majority vote of those voting and present at any annual business meeting.

ARTICLE III – PURPOSE

The purpose of this Association shall be the dissemination of information relating to the diagnosis of animal diseases, the coordination of the diagnostic activities of regulatory, research and service laboratories, the establishment of uniform diagnostic techniques, the improvement of existing diagnostic techniques, the development of new diagnostic techniques, the establishment of accepted guides for the improvement of diagnostic laboratory organizations relative to personnel qualifications and facilities, and to act in a consultant capacity to the United States Animal Health Association on uniform diagnostic criteria involved in regulatory animal disease programs.

ARTICLE IV – MEMBERSHIP

Any laboratory worker engaged in the field of disease diagnosis in animals is eligible for membership.

ARTICLE V – MEETINGS

The meetings of the Association shall be annual and special.

ARTICLE VI – OFFICERS

The officers of this Association shall be: Chairman, Chairman-elect, and Secretary-Treasurer.
ARTICLE VII - EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the Chairman, Chairman-elect, and Secretary-Treasurer. The Executive Committee shall carry out the administrative functions of the Association and shall report its activities periodically, not less than annually, to the Association's membership.

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies.

The Chairman of the Association shall be the Chairman of the Executive Committee.

ARTICLE VIII - PROGRAM COMMITTEE

The Program Committee shall consist of the Chairman-elect and four other members, one each, respectively, from the four districts of the United States, appointed by the Conference Chairman. 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, consisting of 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.

ARTICLE IX - DUTIES OF THE OFFICERS

1. Chairman: It shall be the duty of the Chairman to preside at all meetings of this Association and to appoint all committees. The Chairman shall be an ex officio member of all committees and Chairman of the Executive Committee. (Also see Article VII.)

2. Chairman-elect: In the absence of the Chairman, the Chairman-elect shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the Chairman, he shall perform all duties of the Chairman. He shall be a member of the Executive Committee and Chairman of the Program Committee.

3. Secretary-Treasurer: The Secretary-Treasurer shall keep an accurate record of the meetings of the Association. Whenever authorized by the Executive Committee, he shall publish newsletters and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the meetings of the Executive Committee and shall furnish a copy to each member of said Executive Committee.

He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman a list giving the name and address of each member and an annual financial report. He shall perform such other duties
as may be authorized and prescribed by the Executive Committee. He shall be the Secretary of the Executive Committee, also an ex officio member of the Program Committee.

ARTICLE X – 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.

ARTICLE XI – EXECUTIVE BOARD

The Executive Board shall constitute the legislative body of the Association. Membership of the Executive Board shall consist of one delegate from each state. A member of the Executive Board must be a supervisor of laboratory veterinarians actively engaged in diagnostic medicine and who has been duly designated by the veterinary diagnostic personnel of the state he represents. Alternates may be designated to serve in the absence of regular delegates.

A quorum shall consist of 12 members.

The Board shall report its activities annually to the Association’s membership, and establish its own rules of conduct.

The Chairman, Chairman-Elect, and Secretary-Treasurer of the Association shall be ex officio members. The Association’s Chairman shall act as the presiding officer of the Executive Board and shall cast a ballot in tie votes.

BY-LAWS

ARTICLE I – ORDER OF BUSINESS

Registration.  
Call to order.  
Report of Secretary-Treasurer.  
Chairman’s Address.  
Committee Reports.  
Discussion.  
Unfinished Business.  
New Business.  
Nomination and Election of Officers.  
Adjournment.  

A suspension of the By-laws may be made by a two-thirds majority vote of those present and voting 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.

An individual member may be expelled for cause upon recommendation of the Executive Committee and a majority vote of a regularly scheduled meeting of the membership.

ARTICLE III – MEETINGS

The annual meeting of the Association will be held the two days preceding the dates of the annual meeting of the United States Animal Health Association and at the same location.

ARTICLE IV – QUORUM

Twenty members of the Association shall constitute a quorum.
Two members of the Executive Committee shall constitute a quorum.

ARTICLE V – PROCEEDINGS


(The articles for publication shall be assembled by the Chairman of the Program Committee and forwarded by him to the Secretary of the United States Animal Health Association immediately following each annual meeting.

All articles for publication in the United States Animal Health Association Proceedings shall conform to the style requirements of the Journal of the American Veterinary Medical Association.

Abstracts of the articles to be presented at the annual meeting shall be assembled by the Chairman of the Program Committee and submitted to the Secretary of the United States Animal Health Association prior to thirty days in advance of the scheduled annual meeting dates).

ARTICLE VI – AMENDMENTS

The By-laws may be amended by a majority vote of the members of the Association present and voting at an annual meeting.

ARTICLE VII – ELECTION OF OFFICERS

All elective officers (Chairman-elect and Secretary-Treasurer) shall be nominated by a nominating committee appointed by the Chairman, and by nominations from the floor of the annual business meeting. The terms of office for the above shall be for one year following their election. Interim vacancies shall be filled by appointment by the Executive Committee and such appointees will serve until the next regularly scheduled business meeting.

ARTICLE VIII – DUES

The amount of registration fees and/or dues shall be determined by a
two-thirds majority vote at any regularly scheduled annual business meeting.

ARTICLE IX – STANDING COMMITTEES

The Chairman shall appoint committees when instructed to do so by the Executive Board or, if it is not in session, by the Executive Committee.

ARTICLE X – MINIMUM STANDARDS FOR VETERINARY DIAGNOSTIC LABORATORIES

The Association shall develop minimum standards for the certification of veterinary diagnostic laboratories.
STUDIES ON ACUTE MAREK’S DISEASE
DETECTION OF ANTIBODIES WITH A
TANNIC ACID INDIRECT HEMAGGLUTINATION TEST

C. S. Eidson
Poultry Disease Research Center
University of Georgia
Athens

SUMMARY

An indirect hemagglutination test for detecting Marek’s disease (MD) anti-
bodies in chicken serum is described. Hemagglutination was produced when tanned
horse erythrocytes treated with an antigen prepared from duck embryo fibroblasts
infected with the GA isolate of MD were tested against sera from MD infected
birds. The specificity of the test was demonstrated by the absence of hemag-
glutination with normal chicken serum in the presence of the MD tissue culture
antigen and with normal tissue culture antigen in the presence of a known positive
serum.

The indirect hemagglutination antibody titer of chickens infected with the
Marek’s disease agent (MDA) suggests a direct relationship to the chicken’s ability
to survive the disease.

INTRODUCTION

Marek’s disease is a highly contagious disease of chickens, and the causative
agent is present in blood and tumors. Churchill and Biggs and Solomon et al. have shown that chicken kidney cell cultures inoculated with infected blood or
tumor cells develop a cytopathic effect (CPE) associated with the presence of a
herpes-type virus. Cultures showing CPE result in MD lesions when inoculated into
chicks. These observations led Churchill and Biggs and Solomon et al. to suggest
that the herpes-type virus responsible for the CPE in chicken kidney cultures is the
causative agent of MD. Chubb and Churchill using the agar gel diffusion technique
demonstrated a precipitin reaction with sera from MD-infected birds and antigen
prepared from cell cultures infected with the MD agent and showing characteristic
CPE.

The adsorption of protein extracts of Mycobacterium tuberculosis onto tannic
acid treated red blood cells rendered them agglutinable by specific immune sera. Brown et al. used tanned erythrocytes coated with infectious bronchitis virus to
demonstrate agglutination by specific antiserum. Scott et al. described a hemagglutination method using partially purified herpes simplex virus adsorbed
onto the surface of the red blood cells which had been altered with tannic acid.

This report describes the tannic acid indirect hemagglutination (IHA) test and
gives evidence that the test may be useful in detecting and measuring the amount of
MD-associated antibodies in serum samples from birds that have been infected with
MDA by inoculation or natural exposure.
MATERIALS AND METHODS

Experimental Birds. Chicks were hatched from a random-bred flock designated as Athens-Canadian (A-C) originating with the Southern Regional Poultry Genetics Laboratory, Athens, Georgia.

One-day-old chicks were exposed to MD by intra-abdominal inoculation with 0.2 ml of plasma or by natural exposure. Pooled serum samples from one-day-old hatchmates were checked for MD-associated antibodies. Beginning two weeks after exposure to the MDA serum samples were collected from individual birds at weekly intervals and were checked for antibodies.

Virus. The GA isolate of MD was used throughout this study. Chick kidney monolayers were prepared from 3-to-5-week-old birds injected with a tumor suspension and were inoculated onto infected 2-day-old kidney monolayers at weekly intervals until approximately 20-30% of the cells demonstrated CPE characteristic of the herpes-type virus.

Preparation of Antigen. Duck embryo fibroblasts were prepared from embryonated duck eggs derived from a Khaki-Campbell flock in Georgia. The 12-to-14-day-old embryos were aseptically removed from the shell, decapitated, minced with scissors, washed twice in cold Dulbecco's balanced salt solution, and then trypsinized (0.25%) for 30 to 45 minutes. Blood cells remaining with the trypsinized duck cells were removed by differential centrifugation in an International refrigerated centrifuge. The cells were diluted in growth medium to a final concentration of \(5 \times 10^5\) cells per ml., adding 4 ml to each 60 mm glass petri dish and incubated at 37 to 38°C in a humidified atmosphere containing 5% CO₂.

The growth medium consisted of 199 tissue culture medium with 0.088% sodium bicarbonate and 8% calf serum (heat inactivated) added. One hundred units of penicillin, 100 micrograms of streptomycin and 25 units of mycostatin per ml were added to the growth medium.

The monolayers, complete within 2 days, were then subcultured. This was accomplished with 2 ml of 0.5% trypsin to each petri dish and incubating the trypsin treated monolayers at 37°C for 30 minutes. The cells were removed, washed twice in Dulbecco's balanced salt solution and resuspended in growth medium to a final concentration of \(5 \times 10^5\) cells per ml. Four ml were dispersed into each 60 mm petri dish.

After 24 hours incubation the monolayers were inoculated with \(1 \times 10^6\) infected cultured kidney cells which had been passed 10 times at weekly intervals. After 24 hours the cultures were washed with growth medium to remove excess cells. The infected duck monolayers were passed onto fresh monolayers at biweekly intervals until the CPE was estimated to involve 30 to 40% of the cells.

The cells, removed from the petri dishes by trypsinization, were washed twice with Dulbecco's balanced salt solution, and resuspended to a final concentration of \(6 \times 10^6\) cells per ml in growth medium. Calf serum was omitted at this point to eliminate non-specific reactions.

The cells were frozen (-70°C) and thawed (37°C) 3 times, and the tissue culture antigen stored at -70°C until it was used.

Antisera. Infectious laryngotracheitis antiserum was supplied by the Southeastern Poultry Research Laboratory, Athens, Georgia. Antisera against Newcastle
MAREK'S DISEASE DETECTION

disease virus, the embryo-adapted Beudette strain (42) and the immunizing strain (82828) of infectious bronchitis virus were provided by this laboratory. Antisera against the MD virus were obtained from chickens that had been infected by natural exposure, inoculation with a tumor suspension, direct contact with infected birds or by exposing birds to MD inoculated chicks in the same room in separate batteries. All sera used in this study were heat inactivated for 30 minutes at 56°C.

Saline. A stock solution of 0.85% NaCl was prepared in distilled water. Phosphate-buffered saline (pH 6.4 or 7.2) was prepared by mixing 1 volume of the proper amounts of 0.15 M Na₂HPO₄ and 0.15 M KH₂PO₄ with 19 volumes of saline.

Indirect Hemagglutination Test Procedure. The IHA test is a modification of the technique used by Brown et. al. (2). Horse erythrocytes were collected in Alsever's solution, and were stored at 4°C for as long as 2 weeks. Before use, the cells were washed 3 times in saline. A 2.5% red blood cell (RBC) suspension was prepared in phosphate-buffered saline (PBS) pH 7.2 and incubated 10 minutes at 37°C with an equal volume of 1:20,000 tannic acid which was prepared daily saline. The cells were centrifuged 5 minutes at 1,000 x g, washed once in saline and resuspended in saline at 2.5% concentration. Adsorption of the tissue culture MD-virus antigen was accomplished by incubation of 1 volume of tannic acid-treated RBC with 1 volume of antigen and 4 volumes PBS, pH 6.4, at room temperature for 15 minutes. The sensitized cells were washed twice in PBS, pH 7.2, and resuspended to 0.5% concentration in the same diluent (Fig. 1). Prior to dilution the test sera were absorbed with one-third their volume of normal, packed horse RBC at 37°C for 15 minutes and then packed tightly by centrifugation and the sera decanted. Serial two-fold dilutions of the sera to be tested were made in PBS, pH 7.2. The test was completed by the addition of an equal volume (0.4 ml) of the 0.5% sensitized RBC suspension to each serum dilution. A serum control with untreated horse RBC and a PBS diluent control with sensitized RBC was included (Table 1). The tubes were shaken thoroughly and refrigerated for 3 hours and the results recorded. The agglutination patterns remained stable for 3 to 4 days with refrigeration.

Preparation of MD Immunogen from Photodynamically Inactivated Virus. The MD infected duck cells were trypsinized from petri dishes and resuspended in 199 TC medium (Earle's base) plus 8% calf serum. The cells were frozen in an alcohol-dry ice mixture then thawed at 37°C. Dimethyl sulfoxide (7.5%) was added to the frozen and thawed cells in order to protect the virus particles. Both toluidine blue 0 and neutral red were used to inactivate the MD virus. Toluidine blue 0 was combined in the dark with one portion of the MD virus to obtain a final concentration of 2 ug per ml. The virus-dye mixture was kept at 4°C for 24 hours before irradiation. After irradiation for 1 hour with a day light fluorescent lighting tube the virus was combined with potassium aluminum sulfate (alum) to obtain a final concentration of 12 mg per ml (1:8). A precipitate was formed by rapidly adding a 10% solution sodium carbonate in a volume 2.66 times that of the alum. After settling of the precipitate, the supernatant fluid was removed and the precipitate was suspended in PBS to a concentration one half of the original virus volume (alum - 24 mg/ml).

A second portion of the infected duck cells was treated with neutral red (1:40,000). The procedure was the same as for the toluidine blue 0 dye. Samples
FIGURE 1
A diagramatical flow sheet for the tenning and antigen sensitization of red blood cells.

2.5% washed horse red blood cells in PBS pH 7.2

Mix equal volumes.
Incubate at 37°C for 15 minutes, centrifuge, wash once in PBS, pH 7.2, resuspend to 2.5% RBC.

One Volume antigen
Four volumes PBS, pH 6.4
Mix, incubate at room temperature for 20 minutes.
Centrifuge and wash twice in PBS, pH 7.2. Resuspend RBC to a 0.5% concentration in the diluent and store at 4°C until ready for use.

TABLE 1.
Tannic acid IHA procedure.

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent PBS, pH 7.2</td>
<td>0.6</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Amount of serum diluent mixture transferred</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum dilution</td>
<td>1:4</td>
<td>8</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>1:4</td>
<td>0</td>
</tr>
<tr>
<td>0.5% normal horse cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>0.5% tanned-antigen sensitized horse cells</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
</tbody>
</table>
from the toluidine blue 0 and neutral red treated cells were placed on normal chick kidney cells to determine whether the MD virus was inactivated after irradiation.

**Trial 1:** One-day-old chicks were injected intra-abdominally with $1.5 \times 10^6$ chick kidney cells in order to determine whether inactivated tissue culture cells will protect the chicks against MD.

**RESULTS**

Hemagglutination resulted when an antigen prepared from duck embryo fibroblasts infected with the GA isolate of MD was tested against sera from birds infected with MDA. Duck embryo fibroblasts produced higher yields than chick kidney cells and for this reason were used in the preparation of the tissue culture antigen. Also there would be less chance of incorporating some other chicken antigen into the system. For best results it was necessary to have maximum antigen production in cell culture which was directly associated with the degree of CPE. In the agglutination pattern the cells were deposited over the bottom of the tube with a jagged or irregular edge while negative sera above the 1:8 dilution tubes and the serum and antigen sensitized RBC control tubes were completely buttoned. Agglutination in dilutions of 1:16 or above was considered positive. Horse RBC were used primarily in this study; however sheep erythrocytes gave similar results. Chicken erythrocytes were unsatisfactory for this test.

The specificity of the test was confirmed when serum from uninfected chickens failed to produce hemagglutination in the presence of the MD tissue culture antigen. When the MD tissue culture antigen was replaced with a normal tissue culture antigen in the presence of a known positive serum, hemagglutination failed to take place. MD antigen-treated-erythrocytes did not agglutinate in the presence of laryngotracheitis, infectious bronchitis or Newcastle antisera (Table 2.).

Antibodies were not detected in the pooled sera of one-day-old hatchmates; however, antibodies were detected in the sera of the sires and dams of the parent flock. However, clinical evidence of MD was observed in the parent flock when they were 14 to 20 weeks of age. The isolated control birds remained negative throughout the experiment. In the injected group the disease was so rapid that all birds died by 9 weeks post-inoculation and the antibody titers were relatively low (Table 3). In the room-contact infected chickens, antibodies were detected within 4 weeks. Two of the room-contact chickens died during the 5th and 6th week after they were placed in the room, and the antibody titers of these two chickens were negative. However, the other 3 survivors developed high antibody titers by 13 weeks and demonstrated no clinical signs of MD. Upon necropsy examination no gross lesions were found; however, the kidneys were cultured and CPE characteristic of a herpes-type virus was observed after 5 days.

Table 4 shows the hemagglutination titers of 2 of 25 birds that were injected intra-abdominally at one day of age with a tumor suspension. Twenty of the 25 birds died by the 5th post-inoculation week. One of the two birds used in this study showed no clinical signs of MD by 6 weeks post-inoculation; however, the other bird was listless, dehydrated and had unilateral paralysis. Beginning with the 6th week post-inoculation, serum samples were taken at weekly intervals. By
12 weeks post-inoculation the bird showing no evidence of MD had a hemagglutination titer of 1:1024 while the paralyzed bird had a titer of 1:64. At necropsy examination the clinically normal bird had no lesions and the kidney culture was positive while the paralyzed bird had ovarian and kidney tumors as well as enlarged brachial plexuses, vagus and sciatic nerves.

The incidence of MD lesions in chickens vaccinated with an inactivated virus preparation is shown in Table 5. Both control and vaccinated chicks were challenged with plasma at 3 weeks of age; then they were examined for gross lesions at 8 weeks of age. Table 6 shows that the MD virus was isolated from all birds (control and vaccinated) that were challenged with the MDA-infective plasma; however, it is interesting to note that all vaccinated birds had a positive indirect hemagglutination titer whereas, only one control (challenged) bird had a positive titer (1:16).

DISCUSSION

Chubb and Churchill (3) have reported that it is not known whether the MD-precipitin antigen is viral in origin or whether it is represents a virus-induced cell antigen. Kottaridis et al. (6) have suggested that the antigen could be produced as a result of infection; however, they also postulate that a virus may be present which is capable of revealing more than one antigen in plasma or bone marrow. The MD-tissue culture antigen used in this study also may be a virus-induced cell antigen. However, whether it is viral in origin or a virus induced cell antigen, it appears to be heat stable to 56°C for 30 minutes with only a slight loss in titer to a known positive antiserum.

Chubb and Churchill (3) using the agar gel precipitin test were able to demonstrate maternal antibodies in day-old chicks from antibody positive breeder flocks; however, maternal antibodies were not detected with the tannic acid IHA test. It is not known whether the IHA test is detecting an antibody different from the one in the agar gel precipitin test; however, limited experiments comparing these two tests indicate that serum samples positive for MD-associated antibodies with the agar gel precipitin test were also positive to the IHA test.

The IHA antibody titer of chickens infected with MDA suggests a direct relationship to the chicken's ability to survive the disease (Table 3). Whether the birds were injected with a tumor suspension or were exposed to a contaminated environment, those with a low antibody titer died; whereas those with a high antibody titer survived even though they were exposed continuously to the MD virus. As shown in Table 4, of two birds injected with a tumor suspension, the one which was paralyzed and lived for 12 weeks never had an antibody titer in excess of 1:64, whereas the second bird which appeared normal throughout had an antibody titer of 1:1024. The cultured kidney revealed that the virus was still present in the chicken.

As yet unexplained is the different response in two birds subjected to identical exposure and environment.

The results shown in Tables 5 and 6 support the data in Table 3, indicating that the IHA antibody titer of chickens infected with MDA suggests a direct relationship to the chickens' ability to survive the disease. Although fifteen percent of the vaccinated birds contained gross lesions, they may have been able to survive the disease if they had not been sacrificed.
TABLE 2.

Serum hemagglutination response of various antisera with a Marek's disease tissue culture antigen.

<table>
<thead>
<tr>
<th>Antiseruma</th>
<th>Hemagglutination Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laryngotracheitis</td>
<td>4b</td>
</tr>
<tr>
<td>Infectious bronchitis virus (82828)</td>
<td>0</td>
</tr>
<tr>
<td>Infectious bronchitis virus (Beaudette)</td>
<td>8</td>
</tr>
<tr>
<td>Newcastle disease</td>
<td>0</td>
</tr>
<tr>
<td>Marek's disease</td>
<td>512</td>
</tr>
</tbody>
</table>

aPooled serum samples.
bHA titer of 1:8 or less is negative.

TABLE 3.

Serum hemagglutination response with a Marek's disease tissue culture antigen.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Inoculated birds</th>
<th>Room contact birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 3 4 5 6 7 8 9 10 11 12 13 MD lesions</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>1</td>
<td>0 0 16a 16 16 32 D b</td>
<td>0 0 4 8 D</td>
</tr>
<tr>
<td>2</td>
<td>0 0 16 16 32 32 32 64 128 128 512 512</td>
<td>0 0 16 16 32 64 128 256 512</td>
</tr>
<tr>
<td>3</td>
<td>0 0 0 8 D</td>
<td>0 0 0 0 16 16 16 32 64 128 256 512</td>
</tr>
<tr>
<td>4</td>
<td>0 0 0 8 16 16 32 D</td>
<td>0 0 0 0 16 16 16 32 64 128 256 512</td>
</tr>
<tr>
<td>5</td>
<td>0 0 0 8 D</td>
<td>0 0 0 0 16 16 16 32 64 128 256 512</td>
</tr>
</tbody>
</table>

aSerum hemagglutination titer
bDied
Five isolated controls did not develop MD antibodies during the 13 week experimental period.

TABLE 4.

Serum hemagglutination response of two chickens injected with a tumor suspension.

<table>
<thead>
<tr>
<th>Clinical Appearance of bird</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>Normal</td>
<td>32a</td>
</tr>
<tr>
<td>Paralyzed</td>
<td>8 16 16 32 32</td>
</tr>
</tbody>
</table>

aSerum hemagglutination titer.
### TABLE 5.
Incidence of MD lesions in chickens vaccinated with an inactivated virus preparation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Treatment of cells</th>
<th>No. of cells injected</th>
<th>Challenge&lt;sup&gt;B&lt;/sup&gt;</th>
<th>No. positive/ no. injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>yes</td>
<td>Toluidine Blue 0</td>
<td>$1.5 \times 10^6$</td>
<td>0.2 ml plasma</td>
<td>2/10</td>
</tr>
<tr>
<td>2</td>
<td>yes</td>
<td>Toluidine Blue 0</td>
<td>$1.5 \times 10^6$</td>
<td>none</td>
<td>0/10</td>
</tr>
<tr>
<td>3</td>
<td>yes</td>
<td>Toluidine Blue 0</td>
<td>$1.5 \times 10^6$</td>
<td>killed 4 week</td>
<td>0/10</td>
</tr>
<tr>
<td>4</td>
<td>no</td>
<td>--</td>
<td>none</td>
<td>0.2 ml plasma</td>
<td>7/10</td>
</tr>
<tr>
<td>5</td>
<td>no</td>
<td>--</td>
<td>none</td>
<td>none</td>
<td>0/10</td>
</tr>
<tr>
<td>6</td>
<td>yes</td>
<td>Toluidine Blue 0</td>
<td>$1.5 \times 10^6$</td>
<td>0.2 ml plasma</td>
<td>1/10</td>
</tr>
<tr>
<td>7</td>
<td>yes</td>
<td>Toluidine Blue 0</td>
<td>$1.5 \times 10^6$</td>
<td>none</td>
<td>0/10</td>
</tr>
<tr>
<td>8</td>
<td>yes</td>
<td>Toluidine Blue 0</td>
<td>$1.5 \times 10^6$</td>
<td>killed 4 week</td>
<td>0/10</td>
</tr>
<tr>
<td>9</td>
<td>no</td>
<td>--</td>
<td>none</td>
<td>0.2 ml plasma</td>
<td>8/10</td>
</tr>
<tr>
<td>10</td>
<td>no</td>
<td>--</td>
<td>none</td>
<td>killed 4 week</td>
<td>0/10</td>
</tr>
</tbody>
</table>

<sup>A</sup> = Vaccinated at one day of age.

<sup>B</sup> = Challenged at 3 weeks of age.

### TABLE 6.
Indirect hemagglutination titers of vaccinated birds.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccinated</th>
<th>Challenge</th>
<th>Virus&lt;sup&gt;A&lt;/sup&gt;</th>
<th>IHA titer&lt;sup&gt;B&lt;/sup&gt; (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>0.2 ml plasma</td>
<td>10/10</td>
<td>16-128</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>None</td>
<td>0/10</td>
<td>32-128</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Killed 4 week</td>
<td>--</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>0.2 ml plasma</td>
<td>10/10</td>
<td>0-8</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>None</td>
<td>0/10</td>
<td>0-4</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>None</td>
<td>0/10</td>
<td>16-128</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>None</td>
<td>0/10</td>
<td>16-64</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>Killed 4 week</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>0.2 ml plasma</td>
<td>10/10</td>
<td>0-16</td>
</tr>
<tr>
<td>10</td>
<td>No</td>
<td>Killed 4 week</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>A</sup> = Kidneys were cultured for MD virus.

<sup>B</sup> = IHA titer of 1:16 considered to be positive for MD antibodies.
REFERENCES


THE DIFFERENTIAL DIAGNOSIS
OF MAREK'S DISEASE AND LYMPHOID LEUKOSIS

R. K. Page; O. J. Fletcher and C. S. Eidson

In spite of the many advances in the research of Marek's disease and lymphoid leukosis, the problem for the diagnostician has changed little. The newer knowledge relative to the distinction of these diseases has evolved through the use of costly, time consuming techniques that are impractical for the average diagnostic laboratory. The clinician still must rely on the clinical signs, post mortem lesions and flock history to arrive at a diagnosis. With the advent of controlled environmental housing, the ability to differentiate between these two diseases becomes more important.

With these thoughts in mind let's look at these two diseases from a clinical point of view. Clinical signs of lymphoid leukosis and Marek's disease are ambiguous. Paresis is diagnostic for MD; however, most cases of MD will not have clinical manifestations of paresis. Post mortem lesions of MD and LL can be and frequently are very similar. Enlargement of the Bursa of Fabricius, while considered part of LL can occur in MD. Histologically the Bursal enlargement of LL is an intrafollicular proliferation as contrasted to the interfollicular proliferation of MD. Central or peripheral nervous system involvement with mononuclear cells is suggestive of but not conclusive for MD.

Cytological studies conducted on these diseases have indicated cytoplasmic pyroninophilia and uniformity of cellular size are usually seen with LL, while pleomorphism and variable pyroninophilia are described for MD. Cytological differentiation of these two neoplasms, although possible is very difficult and subject to a great deal of variation in interpretation. If one studies several birds from a given flock many stages of lymphoid cell development will be seen, consequently the interpretation becomes very difficult.

Skin lesions have become a very important part of the Marek's disease picture. So far as presently known skin lesions have not been observed in LL. At the present time skin lesions are responsible for the major portion of the MD condemnations in broilers in the southeast.

Some time ago we became interested in the use of fluorescent antibody for the differentiation of MD and LL. Conjugates were prepared by hyperimmunizing chickens with either tumors of MD or LL. After a series of 5-6 injections with macerated tumor material, these chickens were bled out, the globulin precipitated and conjugated to fluorescein isothiocyanate.

The application of this conjugate to smears, or frozen sections has resulted in some rather unusual findings. In MD tumors there are relatively few cells that show fluorescence.

This fluorescence is both nuclear and cytoplasmic. In contrast the fluorescence observed in LL has been cytoplasmic, giving a halo effect to the cell. The LL tumor is composed of a virtual sheet of these large cells exhibiting this peripheral cytoplasmic stain.

We believe this technique has an application in the diagnostic laboratory for the differentiation of MD and LL. In our hands it is faster, easier to perform and more specific than the techniques, now being used to accomplish this differentiation.
FIELD AND LABORATORY STUDIES
WITH MAREK'S DISEASE

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INTRODUCTION

Marek's disease is a common affliction of the domestic chicken. It is exhibited by lesions in the peripheral nerves, other tissues and visceral organs. There is evidence to support the finding that the etiological agent is a herpesvirus (Witter, Burgoyne, and Solomon, 1968).

Economic losses to the poultry industry and to the food supply resulting from the occurrence of Marek's disease have been measured (Raskopf, 1968). A total of 2.33 per cent of the broilers slaughtered in the United States from 1959 through 1966, or 389,392,000 birds representing a live-weight loss of 1,339 million pounds, was condemned at time of post mortem plant inspection. The condemnation rate was 2.41 per cent from 1961 to 1966. "Leukosis", or Marek's disease, accounted for 17.31 per cent of all condemnations. Broilers under Federal inspection were condemned at a rate of 3.38 per cent in 1959 to 1966 in the State of Maine. Lesions of Marek's disease accounted for 31.9 per cent of the total condemned.

The trend in "leukosis" condemnations in the United States has increased from 0.11 per cent in 1961 to 0.93 per cent in 1966. The condemnation rate for leukemia alone in the United States averaged nearly 1.3 per cent in 1967 and represented about 36 per cent of all condemnation losses.

The results of studies with State of Maine broiler flocks, managed and monitored as Specific Pathogen Free, have shown that certain diseases can be eradicated by stringent adherence to sanitation, isolation, and good management, without the use of various expensive antibiotics and vaccines (Chute, 1964a, 1964b, 1968; Chute et al 1964, 1965). The occurrence of Marek's disease in these

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same flocks has not been reduced. In fact, a trend toward increased occurrence has been obvious as indicated by levels of condemnation at broiler processing plants under the inspection supervision of the Consumer and Marketing Service of the U.S. Department of Agriculture. A summary of the factors which caused condemnations in 319 Main broiler flocks in 1967-68 has been prepared. The 319 flocks represented approximately eight million birds killed in a continuous production period of one year of integrated broiler production. The average condemnation due to disease was 3.94 per cent. Of this total, 62 per cent was due to "leukosis", or Marek's disease (official Specific Pathogen Free Program data recorded at Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, Augusta, Maine, by D. D. King).

Data released March 5, 1969 by the U.S. Department of Agriculture indicated that young chickens slaughtered under federal inspection in January, 1969 were condemned at a rate of 4.21 per cent nationwide (U.S. Department of Agriculture, 1969). During the same period, young chickens were condemned in Maine at a rate of 4.81 per cent. A total of 130,573 birds, or 54 per cent of all condemnations, was due to lesions of Marek's disease in the Maine flocks.

Various means have been attempted to reduce the disease occurrence. These include genetic selection of breeder stock, stringent isolation and sanitation, use of chemical sprays, insecticides, disinfectants, sanitizers, use of antimicrobial agents, i.e.; antibiotics and sulfa drugs, and various degrees of "controlled" exposure, many of which appeared to alleviate the condition, but none of which enabled control of any predictable degree.

The exhibited lesions of Marek's disease evolve from a lymphoproliferative response to the exposure of the etiological agent. These lesions are detectable by gross inspection procedures at the broiler processing plant. They appear as focal accumulations of lymphocytes which form varying sizes of light-colored, pale, white, cream-colored lesions in the spleen, liver, kidney, testicle, ovary, pro-ventriculus, lung, muscle, pancreas, intestine, heart, gizzard, nerves, thymus, skin, eye, and bone. These lesions, occurring singly or in any combination grossly, are evidence of a general systemic disturbance, thereby warranting condemnation of the carcass (Consumer and Marketing Service, 1967).

The need for a study of factors affecting the occurrence of skin and visceral lesions in the broiler during the growing period and at time of processing has been emphasized since August 1, 1967. At that time, the poultry inspectors agreed to record the incidence of skin lesions as well as visceral lesions of broiler-age birds during the inspection procedure on a trial basis. Prior to that date, all condemnations for Marek's disease were grouped into one category: "Leukosis". Variations were then observed in the ratio of skin lesions to visceral lesions as causes of condemnations of broilers. It was determined that 2.43 per cent of the eight million birds processed were condemned for Marek's disease, of which 75 per cent were due to skin lesions of Marek's disease (Specific Pathogen Free Program data). The difference between sexes in skin lesion condemnations were also observed and confirmed histologically (King and Chute, 1969).

This study was undertaken to gain further information concerning Marek's disease as it effects the integrated broiler industry and private broiler growers.

The following objectives were established:
1. To determine if a pattern of onset of lesions does occur in Maine Specific Pathogen Free flocks;
2. To determine the effect of age, sex, and breed upon the pattern, if it does exist;
3. To determine the variation in occurrence of skin lesions of Marek's disease in three cross-mating's progeny as to age and sex; and
4. To further evaluate histologically and grossly, the correlation of skin lesions with visceral and neural lesions of Marek's disease in the broiler-age bird.

MATERIALS AND METHODS

A total of 15,660 day-old broiler chicks was placed in a three-story, thirty-six pen (twelve pens per floor), conventional, uninsulated broiler house which was one mile distant from the nearest poultry farm. All measures specified in routine Specific Pathogen Free cleaning, sanitation, and isolation procedures had been accomplished (Chute, 1968).

The total broiler placement consisted of progeny from three genetically different sources. The chicks had been sexed at the hatchery and were placed in pens of 435 birds each. Placements of sexed cockerels, sexed pullets, and half pullets-half cockerels (representing straight-run placements) were started in the three-pen units (Figure 1.).

At one day of age, one bird was banded and removed from each of the thirty-six pens in the house. This same procedure was followed at 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 days. At time of processing (seven, eight, nine, and ten weeks of age - Figure 2), four birds from each of the thirty-six pens were randomly selected from the dressing line prior to the point where inspection for wholesomeness by the Consumer and Marketing Service occurs. Two birds from those inspected and condemned from each lot were selected.

All birds selected from the house, at the plant, and from the condemned of each lot were identified as to pen of original placement and as individuals. These birds were autopsied and tissue were selected for histological examination. The right sciatic nerve and a section of skin from the lateral aspect of the leg were collected from each bird and identification maintained as to each individual. All tissues were then placed in 10 per cent buffered formalin fixative solution. There they remained for at least 24 hours after which time they were cut, processed in the Technicon time sequence tissue alcohol dehydrator, wax block embedded, microtomed and stained with hemotoxylin and eosin.

All tissues were examined histologically and lesions of lymphocytic infiltration or occurrence of lymphocytes extracellular and extravascular were recorded. In viewing the sections of the sciatic nerves merely the presence of scattered lymphocytes was not considered a positive finding. Either linear, round, or perivascular foci was necessary in order to enumerate the tissue as being positive. The accumulations of lymphocytes in the skin were described as to size, shape, location, and frequency.

All statistical analyses in this study were performed with the aid of the IBM Model 360 Computer.
RESULTS AND DISCUSSION

Reference is made to “breed” as a matter of convenience in discussing broiler progeny performance. Actually, all three broiler multiplier offspring are results of cross-matings.

I. GROSS PATHOLOGY

All birds were processed at the prescribed seven, eight, nine, and ten weeks of age. The total “leukosis” condemnations are summarized for all periods of processing (Table I).

Immediately upon the first period of processing (seven weeks of age), it was noted that a wide variation in skin and visceral lesions was occurring in one complete replicate. Of the three breeds represented, breed number 3 and number 12 had approximately the same proportions of condemned birds, 8.21 and 9.00 per cent, respectively. However, only 0.74 per cent of the 1,230 birds of breed number 10 were condemned for “leukosis”. This extreme gross lesion pattern was of a very short duration for breed number 12, whereby the subsequent replicate processed one week later was incriminated for “leukosis” at approximately the same rate as breed number 10 had been the week previously. The situation for breed number 3 had changed markedly in just seven days, but still showed 3.73 per cent of the flock being condemned for gross skin and visceral lesions of “leukosis”. The high incidence of skin lesions had for the most part subsided after seven weeks of age. Visceral lesions remained at a fairly low rate until a slight upswing occurred at ten weeks of age.

The percentage of each breed condemned for both visceral and skin lesions is noted (Table II). The total percentage condemned for breed number 3 and breed number 12 was very similar, whereas, breed number 10 held consistently lower, except for a late occurrence of visceral lesions at ten weeks of age.

Detection of gross visceral lesions was more consistent than was the skin lesion pattern. The total percentage condemned at each period illustrated how variations of gross lesions within the same broiler house can go undetected, especially where source flock progeny are mixed.

From an analysis of the records of condemnation at seven weeks of age, it was obvious that the pens of pullets had been responsible for most of the skin lesions detected (Figure 3). Here again, the proportions of breeds number 3 and number 12 condemned were very similar, whereas, breed number 10 had a very low incidence of skin lesions. However, no skin lesions were detected from replicate placements three weeks later in breed number 3. Nine week processing data alone would be very misleading in determining the occurrence of skin lesions in broiler progeny. This may explain why mortality date may not be the proper criteria for genetic selection against a problem which is responsible for the majority of broiler condemnations. The pullets placed in sexed groups exhibited skin lesions at a greater rate than did the pullets of the straight-run placements (Figure 4). This may be explained by the epidemiological fact that as a susceptible population is increased the incidence of disease is increased also.
The gross pathology of the cockerels was not as striking as was that of the pullets of the same breeding (Table III). However, it was noted that the highest condemnation for skin lesions was at seven weeks of age. Breed number 3 cockerels experienced the highest incidence of visceral lesions at eight weeks of age. Breed number 10 was only slightly incriminated, except for a somewhat sharp rise in visceral lesions at ten weeks of age. The total performance of breed number 10 cockerels in regard to "leukosis" condemnations was especially good.

No gross lesions of "leukosis" were detected in the nerves of the 500 birds from which sciatic nerves were collected in this study.

II. MICROSCOPIC PATHOLOGY

Sciatic Nerves. Lymphocytic infiltration of the lesion type described as type (2) Edematous-edema between nerve fibers and light infiltration with lymphocytes and plasma cells, was the lesion type encountered in this study (Burmester and Witter, 1966). Unlike the pattern of gross skin lesions, there seemed to be no relationship between the incidence of nerve lesions and sex or breed. The only discernible trend in lesion occurrence was a fairly progressive increase in percent detection as age increased (Figure 5). The lesion type encountered was detectable in nerves from chicks as early as one day of age (Figure 5).

As noted in the description of gross pathology of the sciatic nerves, microscopically detectable lesions were not extensive enough to project any gross change.

Skin Lesions Changes. A variation in detection of abnormal feather follicles occurred among breeds, sexes, and ages of broilers in this study. These lesions resulted in condemnation of the entire carcasses of from 0.33 to 7.64 per cent of the birds processed at seven weeks of age. Variable patterns of lesions were determined from microscopic examination of skin tissues collected. Since no literature reference was available to assist in the interpretation of the variable skin patterns, an attempt was made to establish the significance of each pattern based upon the gross lesions at seven weeks of age. Gross lesions at seven weeks were selected as a working base since the condemnation for skin lesions was at its highest at that time, and that was the earliest processing age of the replicate placements.

First, the skin lesion patterns noted were described as to size of lymphocytic focus; number of foci, location of foci, i.e., which layer of skin and the proximity of the focus to the feather follicle area. Lesion Descriptions and specific criteria were as follows:

A. Few small foci of lymphocytes in the muscle layer of the skin: The skin layers were referred to as epidermal, muscular, and adipose in this study. The latter two actually form the dermal layer which lies just beneath the epidermal layer. The feather follicle seems to take root at the feather bud in the muscular layer of the dermis. As the number and extent of lymphocytic foci that are in close proximity to the feather follicle increase, the gross appearance may be altered and detected as being abnormal. A small focus in this study was defined as having a radiant thickness of from one to five lymphocytes aggregated with a
dermal capillary as its center or associated in close proximity. The condition of few foci was defined as being one to five foci located in the tissue section. The foci may be located in either the muscular layer or the adipose layer or both layers.

B. Few large foci in the muscle layer: Defined as one to five foci being more than five cells thick at their radius, and located in the muscle layer of the dermis.

C. Several small foci in the muscular layer: Defined as more than five small foci in the tissue section, all of which are in the muscular layer.

D. Small foci in the adipose layer: These were defined as foci with fewer than five cells thick at its radius and located in the adipose layer of the dermis.

E. Several large foci in the muscle layer: This lesion was defined as more than five large foci in the tissue section located in the muscular layer of the dermis.

F. Extensive lymphocytic infiltration: This lesion was defined as several large foci in the muscular layer and large foci in the adipose layer.

G. Large foci affecting the follicle: This lesion was defined as large foci adjacent or in close proximity to the feather follicle, either near the feather bud or adjacent to the epidermal fold associated with the feather follicle.

H. Large foci in the adipose layer: This was defined as foci larger than five cells in radius located in the adipose layer of the dermis.

Skin lesion patterns were set as variables to examine the association between the various microscopic skin lesion patterns and the gross lesions at time of processing at seven weeks. The incidence of gross lesions at seven weeks expressed as number of birds condemned from each pen was the comparative variable. Microscopic lesions were recorded on statistical tables recording a positive finding as one and the absence of each pattern as zero for each observation. One observation represents the finding of lesion patterns described in each bird, randomly selected from the pens at the time of processing at seven weeks of age and during the growth period. Simple correlation coefficients were determined for each variable.

An assigned value was given to each variable to express the apparent progression of the lesion patterns graphically. This value ranged from one to eight, determined by the ranking of the correlation coefficients at seven weeks of age.

By adding the skin lesions scores of four birds from each pen of sexed or straight-run replicates it was possible to evaluate each breed as to the occurrence of comparable weighted numeric values at each age studied. A comparison among male, female, and straight-run placements was made. These values were graphed to show the variations which resulted (Figures 6, 7 and 8).

Combined scores of sex placements are illustrated for all three breeds (Figure 9).

It can be readily seen that even though breed number 10 had almost a total absence of gross skin lesions at time of processing, a definite pattern of microscopic lesions was obvious (Figure 7). This comparison is substantiated by
analysis which shows breed number 3 and number 12 to have a common lesion pattern, similar to the pattern of gross lesions detected at seven weeks of age (Figure 9). In addition, as shown, there appears to be a period at which the microscopic lesion pattern of breed number 10 peaked (Figures 7 and 9). This peak occurred at three to four weeks of age, after not having reached the magnitude of microscopic lesions as did breeds number 3 and number 12 approximately three weeks later (Figures 6, 7, 8, and 9). This type of graphic analysis suggested that regression of the lesion pattern must have occurred prior to seven weeks of age in the case of breed number 10.

Results illustrated by Figure 9 indicate that even though the peak reached by breed number 10 was lower than breeds number 3 and number 12, the occurrence trend increased at a greater rate. Breed number 10 pullets hit a definite peak in lesion score at three weeks of age and then gave way to an equally definite decrease (Figure 7). The straight-run pens peaked one week later, then decreased steadily. The male pens were more erratic in their lesion pattern but also showed tendencies to decrease before seven weeks of age. These data are inconclusive, but may indicate that the presence of the pullet enhances a more rapid onset as indicated by the lesion score to the extent that a peak of lesions is reached sooner. As a result, the decreased pattern becomes obvious earlier. If this type of pattern could be manipulated throughout the industry, Maine alone would realize more than one million dollars annually from the production of broilers above the present gross income.

As cited earlier, regression of lesions must be considered a probable explanation of the absence of gross skin lesions in breed number 10 at seven weeks of age. With the acute drop in detection of gross lesions in breeds number 3 and number 12, it seems logical to consider regression of lesions among the three breeds.

Lesion changes described up to this point have been restricted to progressive lesion patterns. This was done to avoid confusion as to the correlation coefficient rankings and the graphic expression of the determined lesion scores. However, it was noted early in the study that degrading or regressive lesion changes were occurring.

Death of cells is characterized by changes in the nucleus and cytoplasm and in the cell as a whole (Smith and Jones, 1958). The changes viewed in this study were those of pykonsis, cytoplasmolysis, and loss of cell outline.

These changes involved the cells which were described in the progressive lesions. A description of the regressive lesion pattern is as follows:

A. NC - No change noted in the lesion as to degradation.
B. DSC - Dark staining cells, i.e., varying proportions of lymphocytes which make up the lesion take on a darker staining effect.
C. CDSC - Clumping of dark, stained cells. Lymphocytes comprising the lesion lose their even, homogeneous distribution and tend to clump in groups of cells within the original focus.
D. CBDS - Cells broken down or shrunken. Lymphocytes comprising focus tend to shrink and darken, their walls changing from convex to concave.
E. BD-LD - Breakdown given way to less densely organized focus of lymphocytes.
F. SSDC - Scattered shrunken dark cells. Lymphocytes no longer organized in focal arrangement.

G. OSDC - Only a few scattered dark shrunken lymphocytes remain in lesion.

These lesions are listed in the apparent order of regression as observed microscopically. To determine graphic changes in the lesion, a weighted score was awarded to each lesion described. The value awarded to each lesion as it occurred is as follows:

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. NC</td>
<td>0</td>
</tr>
<tr>
<td>B. DSC</td>
<td>1</td>
</tr>
<tr>
<td>C. CDSC</td>
<td>2</td>
</tr>
<tr>
<td>D. CBDS</td>
<td>3</td>
</tr>
<tr>
<td>E. BD–LD</td>
<td>4</td>
</tr>
<tr>
<td>F. SSDC</td>
<td>5</td>
</tr>
<tr>
<td>G. OSDC</td>
<td>6</td>
</tr>
</tbody>
</table>

The values assigned to the lesion patterns were computed for each breed. A further breakdown was possible by considering the male, female, and straight-run pens within each breed separately. The difference in the progressive and the regressive lesion scores should furnish a graphic expression that would somewhat parallel gross lesions. The lesion differences for all three breeds plotted on the linear graph summarize gross changes which occurred in this study (Figure 10).

The ages at which broilers were processed in this study were chosen to stay within the practical limits of a marketable product for the broiler industry. Many broilers are processed at approximately seven weeks of age which are marketed as a gourmet product referred to as the “Cornish Hen”. Light to heavy broilers encompass the eight to ten week ages at processing. Therefore, even though birds were processed according to the experimental design, there was little sacrifice of wholesome broiler carcasses. A study such as this could only be initiated with the consent and cooperation of the broiler producing companies and the supervisory personnel of the Consumer and Marketing Service.

The higher condemnation for skin lesions at the earlier age processed was not surprising since observations in the field with the Specific Pathogen Free Program had indicated this tendency (Maine Specific Pathogen Free Program data).

The variation among breeds as to gross skin lesion pattern must be explored further. The onset and progression of lesions, documented by histological examination, should add to the overall understanding of the pathogenesis of skin lesions. The fact that the onset and progression pattern of microscopic skin lesions was not directly associated with the sciatic nerve lesions detected may suggest that to refer to the skin lesions as Marek’s disease may be somewhat ambiguous. The above fact coupled with the finding that the total pattern of onset, progression, and regression of microscopic skin lesions in no way paralleled the occurrence of visceral lesions, except that the two conditions occurred in the same flock, does make it seem possible that the causative agent of skin lesion pathology may be separate and apart from the etiological ageny of visceral lesions. In previous studies by other investigators, an association was found
between skin lesions as a facet of visceral lymphomatosis (Jungherr, 1949; Benton, Cover, and Krauss, 1962). It may have been that the susceptibility to the two conditions was similar. In this study, only three broilers were found to have both skin and visceral lesions grossly at time of processing. Perhaps the primary reason for skepticism concerning the skin and visceral lesion association is that upon observing the microscopic skin lesion pattern it was very apparent that the possibility of manipulating the pattern presented itself. Since the presence of gross skin lesions differs from the absence of lesions in infected flocks, only by the degree or extent of the original infection pattern and especially the stage of the pattern, whether progressive or regressive, the aesthetic value of whether the carcass is passed or condemned is the only fact satisfied. Therefore, consideration should be given to the re-evaluation of the criteria for condemnation of "skin leukosis" carcasses. It would seem to the authors that carcasses with detectable gross skin lesions as the only exemplification of disease should be skinned and further processed for human consumption. This may seem to be a drastic recommendation to some. However, for those who prefer to think of "skin leukosis" as being a facet of visceral lymphomatosis, results of studies performed by workers in Massachusetts indicated that the incidence of Type II leukosis (Marek's disease) varied from 25 to 80 per cent of each flock sampled in the field in the Northeastern United States (Sevoian, 1968). Thus, it may not be assumed that infected and uninfected birds at time of processing can be sorted out on the basis of gross lesions only.

The foregoing discussion was directed to the immediate problem of skin lesion condemnation. The skin lesion pattern is at least as important. To be able to manipulate the pattern to "fit" around the processing age would be extremely important in reducing the "skin leukosis" condemnations. The results of this study in which the extent of gross and microscopic lesions were determined (Figures 4, 6, and 8) suggest that the pullet is more susceptible to the skin lesion pathology than is the cockerel. It does seem that density of the pullets may be a factor which may cause a more rapid infection of a greater number of individuals and thereby permits the pullet to reach the peak of the pattern of lesions earlier (Figure 7). It is suggested that the greater the density of pullets the more clearly is the lesion pattern formed. Degree of exposure to the etiologic agent would be greater if the pullet actually sheds the agent more readily and at a greater concentration. With this reasoning, it may be suggested that factors such as ventilation of the broiler house at the early susceptible age should be considered in regard to the density of the causative agent. Perhaps the trend toward increased ventilation has served to dilute the immediate environment of the broiler chick, preventing an optimum degree of exposure whereby the skin lesion pattern is extended into the age of processing. Perhaps this is not the cause even though there is no evidence to refute it. However, degree of exposure should receive uppermost consideration in further work in the manipulation of the skin lesion pattern. For instance, in a controlled exposure program it may be possible to determine factors which affect the progression, peak, and regression of lesions. This, of course, would not be an eradication program, but if measures were found that were capable of predicting the skin lesion pattern, the broiler industry would accept such a program with equal enthusiasm.
Results involving the relationship of sex to skin lesions suggests that the pattern of skin lesions may be affected by gonadal hormone secretions (Benton, Cover, and Krauss, 1962; Helmboldt, Wills, and Frazier, 1963; King and Chute, 1969). In a study performed at the National Institutes of Health, National Cancer Institute, Bethesda, Maryland, it was determined that gonadal hormones affected the susceptibility to skin tumorigenesis (Bates, 1968). Ovarian hormones have been shown to affect the metabolism of nucleic acids, proteins, and phospholipids (O'Malley and Kohler, 1968; Mueller, 1968).

In view of the regression of lesions observed in this study, it is suggested that an "immune response" may be responsible for the degradation of lesions. In experiments with lymphocytic choriomeningitis in mice, it has been demonstrated that a viral infection can result in an immune conflict which can lead to a fatal immunologic disease (Hotchin, 1962). It has been postulated that increased gamma globulin could enhance the pathologic effects of the Marek's disease agent or could result in immunologic injury to target tissues rather than provide protective action (Akhtar, 1966). The presence of the regression pattern emphasizes the dire need for further study to sort out the various lesion patterns referred to in the literature as Marek's disease.

If it can be determined that the regression of lesions is due to an immune response the same principles could possibly be applied in studying the tumorigenic patterns in higher animals.

SUMMARY

A broiler flock of 15,660 birds composed of equal proportions of progeny from three genetically different multiplier flocks, was randomly sampled during the growing period and at the time of processing at seven, eight, nine, and ten weeks of age. Gross lesions were recorded and tissues collected from each bird included a sciatic nerve and a section of skin from the lateral aspect of the leg. These tissues were examined histologically for the presence of lesions indicative of Marek's disease. A total of 486 birds was subjected to histological examination.

Gross condemnations at time of processing at seven weeks of age of the first of four replicate placements in the house indicated that variations occurred among breeds and sexes of placements. The variation in condemnations among breeds due to skin and visceral lesions of Marek's disease ranged from 0.73 per cent to 9.00 per cent. Condemnations due to skin lesions ranged from 0.33 to 7.64 per cent. By eight weeks of age skin and visceral condemnations had decreased to a range from 0.08 per cent to 3.70 per cent among the three cross-matings. By nine weeks of age the range for total skin and visceral condemnations had decreased to 0.24 per cent to 0.98 per cent. At the final processing of the four replicates at ten weeks of age the range for skin lesion condemnations had decreased to a range of from 0.00 per cent to 0.89 per cent. The greatest change had been due to the decrease in gross skin lesions. The greatest number of skin lesions had been detected in the pullets, especially those from the sexed pullet pens, as opposed to the cockerel and the straight-run placements.
One of the cross-mating studied consistently showed a low incidence of lesions indicative of Marek's disease.

The microscopic examination revealed the onset, progression, and regression of lesions in the skin which was shown to correlate with the gross lesions detected. The lesion pattern varied and it was shown that the absence of gross skin lesions at seven weeks of age in the one broiler progeny was due to a regression pattern which had progressed adequately so that gross lesions were not detectable. Similar findings accounted for the decrease in the incidence of skin lesions of the other two cross-matings at subsequent periods of processing. Pullets were found to be more extensively infected as determined by the microscopic lesion pattern throughout the growth period prior to the first processing period at seven weeks of age.

The pattern of skin lesions grossly or microscopically did not correlate with the visceral or nerve lesion patterns.

Even though the skin lesion pattern varied among breeds, the nerve lesion pattern was fairly consistent among all breeds and no difference among sexes was noted in the nerve lesion pattern.

**CONCLUSIONS**

1. Wide variations in detectable gross skin and visceral lesions occurred among breeds and sexes in a similar environment.

2. Pullets were incriminated grossly more frequently than cockerels for skin lesions.

3. The microscopic lesion pattern of the skin was one of onset, progression, and regression. The extent and the stage of the pattern intersected at time of processing accounted for the varied levels of condemnation. The peak of skin lesions detected microscopically in one breed occurred as early as three weeks of age.

4. Nerve lesions detected in this study did not correlate in pattern with the skin lesions. There was some tendency for the nerve lesions to parallel the occurrence of visceral lesions.

5. Nerve lesions were detectable in chicks one day of age field conditions.
<table>
<thead>
<tr>
<th>No. of Birds*</th>
<th>Breed</th>
<th>Sexed Pullets</th>
<th>Straight-run pens</th>
<th>Total Pullets</th>
<th>Sexed Cockerels</th>
<th>Straight-run pens</th>
<th>Total Cockerels</th>
<th>Total Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>4920</td>
<td>3</td>
<td>68</td>
<td>8</td>
<td>82</td>
<td>16</td>
<td>23</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>4920</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>10</td>
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<td>19</td>
<td>26</td>
<td>4</td>
<td>23</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>138</td>
<td>49</td>
<td>182</td>
<td>64</td>
<td>28</td>
<td>50</td>
<td>18</td>
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*Each cross-mating composed of 4920 birds consisted of 1640 birds each in the sexed pullets and sexed cockerels groups. The pullets from the straight-run placement and the cockerels from the straight-run placement each accounted for 820 birds or a total per placement of 1640 birds.
TABLE II

PROPORTION OF EACH BREED CONDEMNED FOR BOTH VISCERAL AND SKIN LESIONS (PER CENT)*

<table>
<thead>
<tr>
<th>Breed</th>
<th>7 Weeks of Age</th>
<th>8 Weeks of Age</th>
<th>9 Weeks of Age</th>
<th>10 Weeks of Age</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>8.21</td>
<td>3.70</td>
<td>0.89</td>
<td>0.65</td>
<td>3.37</td>
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<tr>
<td>10</td>
<td>0.73</td>
<td>0.08</td>
<td>0.98</td>
<td>1.38</td>
<td>0.79</td>
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<tr>
<td>12</td>
<td>9.00</td>
<td>0.81</td>
<td>0.24</td>
<td>2.03</td>
<td>3.03</td>
</tr>
<tr>
<td>Total</td>
<td>8.00</td>
<td>1.54</td>
<td>0.70</td>
<td>1.08</td>
<td>2.40</td>
</tr>
</tbody>
</table>

*1230 birds from each breed were processed at each age.
TABLE III

TOTAL NUMBER OF COCKERELS CONDEMNED FOR "LEUKOSIS" BY LESION PATTERN

<table>
<thead>
<tr>
<th>Breed</th>
<th>7 Weeks of Age</th>
<th>8 Weeks of Age</th>
<th>9 Weeks of Age</th>
<th>10 Weeks of Age</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin Visceral</td>
<td>Skin Visceral</td>
<td>Skin Visceral</td>
<td>Skin Visceral</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>10</td>
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<td>2</td>
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<tr>
<td>10</td>
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<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>4</td>
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<tr>
<td>Total</td>
<td>29</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>46</td>
</tr>
</tbody>
</table>

*615 cockerels were processed at each age for each of the three breeds.
3, 10, 12 = units of cross-matings
7, 8, 9, 10 weeks = age at processing
M, F, SR = Male, Female, Straight-run

Pen size = 435 birds
Unit = 1305 birds
FIGURE 2

PATTERN OF BROILER PICK-UP AT TIME OF PROCESSING
FIGURE 3
TOTAL PULLETS CONDEMNED FOR "SKIN LEUKOSIS"
FIGURE 4

PULLETS CONDEMNED FOR "SKIN LEUKOSIS" FROM SEXED AND STRAIGHT-RUN PLACEMENTS"

*Values for straight-run have been adjusted by multiplying by two since the pullets from the straight-run pens represent only one-half of the total pen placement.
SCIATIC LESIONS DURING GROWTH PERIOD FOR ALL BREEDS

FIGURE 5

Breed Code
3
10
12

Age

FIGURE 5

SCIATIC LESIONS DURING GROWTH PERIOD FOR ALL BREEDS
FIGURE 6
WEEKLY SKIN LESION SCORES FOR BREED NUMBER 3
STUDIES OF MAREK'S DISEASE

FIGURE 7
WEEKLY SKIN LESION SCORES FOR BREED NUMBER 10
FIGURE 8
WEEKLY SKIN LESION SCORES FOR BREED NUMBER 12
FIGURE 9
WEEKLY SKIN LESION SCORES FOR ALL BREEDS
FIGURE 10
MACROSCOPIC SKIN LESION DIFFERENCES FOR ALL BREEDS


Welcome to the Annual Conference of the American Association of Veterinary Laboratory Diagnosticians.

The program committee this year considered the topic of the educational needs of diagnostic veterinary medicine to be of utmost importance, and so we feel extremely fortunate in having been able to assemble the distinguished panel of experts seated before you to discuss this topic in depth.

The problem and questions related to the future needs of biomedical scientists engaged in diagnostic medicine are:

**THE PROBLEM**

The current changes in the livestock industry and future changes required to meet the needs of a rapidly expanding human population for food is demanding more precise diagnoses of animal diseases. Clinical diagnoses without laboratory confirmation is rapidly becoming obsolete. A diagnosis based only on man’s senses or without regard to herd impact has become archaic. Diagnostic medicine is no longer an art; it is now a science being applied by veterinary private practitioners, laboratory diagnosticians, management consultants, regulatory specialists and research personnel.

**QUESTIONS**

1. What kind of training is needed to make each of these groups effective practitioners of the science of diagnostic medicine in their specialty areas?
2. What changes must be considered in future training programs:
   a. undergraduate,
   b. graduate,
   c. continuing education?
3. How to orient and educate nonlaboratory groups in the effective utilization of laboratory medicine in the health management of herds as well as the historical concept as a guide to treatment.

I’m sure our panel participants will have additional problems and questions to pose, and hopefully some answers.
Dr. Lyle, Ladies and Gentlemen:

It is a pleasure to be here. I appreciated Dr. Lyle's invitation to appear on your program. I am not learned in this field, but I am interested. Dr. Lyle mentioned that the developments in this field of the application of veterinary medicine has progressed to the point that it may now be called a science rather than an art. This may not be precisely the way you said it, Dr. Lyle. The last time I looked in a medical dictionary, diagnosis was listed as the art of distinguishing one disease from another. I would submit that Dr. Lyle is absolutely correct in dwelling upon the advancements in the science, but I would suggest that it remains in name both an art and a science. If you work on your statement, I'll work on the dictionary, Dr. Lyle.

Diagnosis is the discovery of the nature and the source of a patient's abnormality. There's a New York case in which the court says that a diagnosis is "said to be a little more than a guess, enlightened by experience." Well, we have progressed a long way from that point of view. I think this case emphasizes the point that seems to me important, that there is a great deal of art brought into the making of a diagnosis, and that this derives in large part from experience.

Now what's the purpose of making a diagnosis? It is to prescribe treatment or surgery; it is to guide epidemiological investigations; it's to guide efforts to prevent the spread of disease; it is to guide control or eradication efforts; it is to provide background for public health protection; it may be just to provide another chapter in the volume of information that we have on diseases; or it may be to transmit information to students — graduate or undergraduate.

Who makes the diagnosis? It is because of some conversations I had on this point, I think, Dr. Lyle, that I happen to be here. I ventured to say that the name of this organization was incorrect — that the name "American Association of Veterinary Laboratory Diagnosticians" is for the most part not correct, because its members do not diagnose.

You furnish expert information upon the basis of which someone else makes the diagnosis, unless you are both clinician and laboratory scientist. I find that on this matter there are differences of opinion. There is confusion that gives rise to problems especially for the practitioner.

Most practitioners are happy to send material to the diagnostic laboratory and to receive in return "a diagnosis." This, it seems to me, is an illogical attitude on their part, for they really are searching for information. They themselves must necessarily assume the responsibility for making the diagnosis. But, it's common practice to refer to the results of tests as being diagnostic especially when one is in a bit of trouble about the diagnosis.
The practitioner or other clinician has to assume responsibility for the diagnosis. He should look to the laboratories for very essential services to help him in reaching the right conclusion. Misunderstandings arise when the scientist in the laboratory designates his reported findings as a "diagnosis." That is wrong, unless he also is a clinician. I hope that improvements in terminology may be worked out to better serve both groups. It has occurred to me that the use of a term such as "Laboratory Services Veterinarians" rather than "Veterinary Laboratory Diagnosticians" might be a step in the right direction. I suppose that change would not be accepted with enthusiasm, Dr. Seaton.

This has come to my attention primarily because of the work of the AVMA Professional Liability Insurance Trust. This excellent program provides the best malpractice insurance available to veterinarians, primarily because the group of veterinarians who are the trustees insist, with the full concurrence of the insurance company and the administrator, that the veterinarian be defended when he is right regardless of the cost. They have refused many times to settle a case that could have been settled for a small sum and have spent much more in defending the actions taken by the veterinarian. This is not because of the individual, but for the protection of the veterinary profession. Such actions have a sobering effect on others who may be contemplating malpractice suits against other veterinarians.

It has become important on several occasions to know precisely who made the diagnosis and upon what basis it was made. It has been stressed upon me as being of key importance that there be no misunderstanding as to who makes this determination. It is the person who had the animal under his professional care who must make the diagnosis and must act in accordance with it.

Perhaps some of our needs in continuing education would be served if arrangements were made for laboratory scientists and practitioners to get together more often for discussions of problem cases. I had the opportunity to see one such effort at the Purdue conference. I was impressed with the fact that the views expressed by the laboratory scientist, the practitioner, and the university clinician had to be explained further and in some respects modified in order to reach a consensus. Such panel discussions are useful.

Dr. R. J. Kolar, who has recently been charged with AVMA responsibilities in continuing education, will be giving attention to the desirability of that kind of cross fertilization of ideas and instructions. You have done a great deal in the last few years to upgrade your own competence. It has been very noticeable that you have been giving close attention to the monitoring of laboratory procedures, and for this we should all be grateful. This is not an easy task.

Many State Veterinarians are concerned about how they should upgrade laboratory service facilities, procedures, and records. I want to assure you of every support from the AVMA as you continue to work on this very difficult and important problem.
TRENDS FOR POULTRY LABORATORY DIAGNOSTICIANS

A speech presented at the AAVLD, Milwaukee, Wisconsin
October 13, 1969
by
H. L. Chute, D.V.M., University of Maine,
Orono, Maine

Dr. Lyle, Ladies and Gentlemen:

As I speak here relative to diagnostic veterinary medicine, I speak only, I guess, from my experience in the poultry field, which is of course somewhat different than what Dr. Clarkson had reference to in his address.

We in poultry diagnostic work are a very unique group in that our type of work probably is somewhat different than what the general practitioner has to do, the small animal practitioner, or other facets of veterinary medicine. And I think that generally we’re fortunate as veterinarians because we have the capability and need for nearly every industry for the use of our profession. In other words, we can get into all kinds of things and it’s just that our heritage has been associated with agriculture, which has fallen into a giant now called agricultural business or agri-business, but with tremendous duty opportunities for veterinarians.

Some say that twenty-five or thirty years ago we lost all poultry practice due to a lack of interest. However, I think today we have a great many veterinarians who make a full time livelihood from poultry diagnosis and poultry business in more than one way. Actually there are probably thirty or maybe fifty times more veterinarians today making a living in connection with poultry than thirty years ago. In the State of Maine about 25 per cent of the veterinarians are engaged in full time poultry work, which includes diagnosis, research, regulatory and inspection. It is not difficult to imagine that this same trend will continue to grow to include animal practice in all phases of veterinary medicine.

Sometimes we as veterinarians are our own worst enemies, trying to do things that we aren’t qualified to do. Of course that may reflect on the panel this afternoon. But I think what you well realize is that we must have, or will have, in the very near future, an almost total specialization in various activities, and will then by necessity be decreased in scope, and I would hope markedly increased in intensity and professional competence. The changes we have seen and are seeing in food animal practice may not be very good for some veterinarians, but I can honestly say that the change is good for the total of veterinary medicine. This evolution eventually will remove the labor factor from practice. Not the sweat and worry, but the labor, so our duties and responsibilities will continue to change and to evolve. It is our duty to be flexible enough in our thinking and our actions to keep ahead of change and to help plan for it rather than trying to compensate as a result of change. So far we have been successful. We need to thank our predecessors for their actions. Even the rank pessimist can conceive a great potential for diagnostic veterinary medicine if we keep our capacity to bend and to change as changes take place.

Besides the undergraduate training necessary in, say, poultry diagnostic practice, of course the six years are essential in veterinary medicine and we need to
encompass all types of courses of training. The basic technical courses are adequate. However, a diagnostic veterinarian in poultry has to have a knowledge that is quite specialized. For instance, he must have an excellent knowledge of poultry, he must have a good knowledge of poultry production, he must have a current knowledge of poultry research—what’s happening in the field. He must be able to differentiate between Marek’s and lymphoid leucosis and all other types of neoplasms. He must have a good knowledge of poultry inspection. He must have a knowledge of public relations or he cannot exist in the area of poultry diagnostic work. He must have a knowledge of the food and drug administration, because this is so vitally involved in the poultry field.

I refer to the American Association of Avian Pathologists (AAAP) because they have set a standard of three years of poultry disease experience as necessary for membership in that organization. They suggest these minimum requirements after a degree in veterinary medicine but they also recommend an advanced degree for study in pathology, microbiology, or other fields as designed. The high degree of sophistication in poultry diagnostic work today is self evident. We need a highly skilled knowledge of gross, and microscopic pathology, associated with the clinical syndromes involved in large poultry operations of flocks twenty-five thousand or larger. In Maine, which is ninth in the country in poultry production, in the broiler industry, we are not building a broiler house now that holds less than sixty-thousand birds. That’s a long call from thirty years ago when we had a flock of one hundred hens.

You not only must have a broad knowledge of laboratory work, but you must know something about the clinical syndrome associated with these diseases, because this is probably different than you were ever taught in school. Besides this, the laboratory must be adapted to all types of disciplines—serology, such as serum neutralization tests, HI tests, HA tests, and this morning you heard of another one for Marek’s disease. It is now becoming very common practice in all poultry diagnostic laboratories to use fluorescent antibody techniques. In fact, they are becoming so common that every laboratory is essentially getting into this area. Therefore, how can we possibly train our staffs, not to mention the laboratory directors in all of these new techniques?

Again, I refer to a report by the American Association of Avian Pathologists on continuing education. Dr. Clarkson mentioned continuing education but the AAAP is fairly active in this, and this report was presented at the last AMVA meeting in Minnesota in July by Dr. Tumlin from Georgia who was chairman of this committee. Their report pointed out, or brought to the floor, the courses that are being given the next year or two insofar as continuing education for poultry disease diagnosis is concerned. I’m not going to give you all the details of this but there have been five short courses, just to give you a background, on fluorescent antibody (FA) technique by Dr. Gentry of Pennsylvania State University. There was a total of twenty-five people who participated in this FA course, which was held at two and a half day periods. The fee of fifty dollars per person was changed for participation in these short courses. As a result of the survey made by the committee, the following veterinary colleges and universities have indicated what they are going to do in this area in the near future. Cornell University is preparing a two and a half day workshop in the preparation and use of cell culture with avian
viruses. They expect to hold one workshop in October and one in November 1969 for a period of two and a half days and these will include sixty persons. The University of Missouri plans to hold, in late 1970, a two day workshop in bacteriology and histopathology. Illinois and Purdue Universities are planning a joint workshop in 1970 on avian virological techniques, the differential diagnosis of viral diseases and possibly histopathology. No date has been set for these courses. The University of California is planning a two day workshop in virology and avian mycoplasmosis sometime in 1970. The following schools have indicated an interest in putting on workshops for the AAP membership. These include the Ontario Veterinary College, in the area of poultry virology, tissue culture, and fluorescent antibody techniques. Michigan State University has expressed desire to cooperate, but want a cooperating program to prevent undue duplication of effect by various schools and colleges of veterinary medicine. The University of Georgia is in a position to establish workshops in virology, tissue culture techniques, fluorescent antibody techniques, bacteriology and histopathology for the southeastern region of the United States.

Again, it is quite different from a general diagnosis. It has great economic impact. In our state almost every integrated poultry operation has a veterinarian working for them and we in universities cooperate with these people. Really, all we should get at the universities are the sophisticated problems that are research problems.
Veterinary college faculty members who teach clinical subjects are in the most advantageous position for setting standards and promoting improvements in diagnostic veterinary medicine. Although clinical medicine has been considered to be as much art as science, there is need for a stronger emphasis on the scientific approach to diagnosis. Without accurate diagnoses, treatments are empirical and the results of therapy are equivocal. In addition to a solid undergraduate education and good clinical experience, clinical teachers must have advanced training, preferably in a residency program. Subsequently, they must be given the time to keep up-to-date with advancements in knowledge and to engage in clinical research. They must keep in close contact with the teachers and researchers in the pre-clinical disciplines. This type of clinician will make full use of available diagnostic services and will promote the development of those which are not available.

A well-trained clinician will have a thorough knowledge of the various laboratory tests and will be able to interpret the results in the light of the case history and his clinical observations. Laboratory tests should be used to confirm or refute a specific clinical diagnosis. Therefore, a clinician should request specific tests for this purpose and not use the laboratory for the purpose of sifting various specimens in the hope of discovering a diagnosis.

The factor of greatest importance in training students in scientific diagnosis is the example set by the clinical teachers. They can encourage or discourage the proper use of laboratory tests by the students. Their approach to diagnostic problems should serve as a guide to the students in the scientific handling of patients. Clinical faculty members are thus in a very influential position. They have many contact-hours with students in relatively small groups and are responsible for completing the undergraduate teaching program. The student is in the stage of his undergraduate education where he is ready to apply his pre-clinical training to the diagnosis and treatment of clinical cases. The laboratory diagnostician is not as influential as the clinician in teaching students the scientific approach to diagnostic problems because he is not usually working directly with the field problems. The laboratorian provides service to the clinicians and can only encourage and support the clinician. The clinician plays the major role in shaping the professional destiny of the students.

Advanced Training in Clinical Studies

A recent graduate who desires to become a clinical teacher needs additional training and experience beyond that obtained in his undergraduate program. We believe that this can be accomplished most satisfactorily by internship and
residency programs. In our experience, Ph.D. programs have frequently failed to produce clinical teachers. Many students have entered graduate programs with the objective of preparing for clinical teaching and research but have failed to accomplish their objective. They usually become side-tracked into a narrow research problem, burdened with course and language requirements and become completely severed from clinical activities. Instead of returning to clinical sciences, many remain in a pre-clinical specialty because of stereotyped graduate training programs to which they were subjected. The appointment of practitioners to teaching positions, without providing them with additional basic training, usually does not provide the necessary caliber of clinical teachers. The goal of the advanced training programs should be to develop the teaching clinicians that are needed. The first step should be to drop the concept of graduate training and replace it with residency training. Placing an adequately motivated student in a favorable environment which encourages an intellectual approach should be more effective than sending him to a laboratory to work on a Ph.D. thesis.

The residency program should also be available to individuals who are interested in laboratory diagnostic medicine. As with clinicians, Ph.D. programs frequently discourage pathologists and clinical pathologists from entering the general field of diagnostic service. The students concentrate on relatively narrow projects and are not afforded the opportunity to obtain the broad level of training which is necessary for diagnostic work. The dire shortage of competent clinical teachers and investigators attests to the face that something is wrong with our present educational system at the post-graduate level.

Continuing Education

Clinical teachers also play a key role in continuing education. The educational needs of practitioners are usually brought first to the attention of staff clinicians in veterinary colleges. Therefore, they are in a position to know what is needed in the field and to influence what is done in the field. They should play a major role in the development of programs for continuing education with the assistance of specialists in the pre-clinical sciences. Extension veterinarians play a key role in continuing education and should be members of clinical departments.

The role of the clinical teacher has been stressed because he is in the key position of influence concerning the proper use and promotion of clinical laboratory medicine.
VETERINARY DIAGNOSTIC LABORATORIES
AND THEIR ROLES IN TEACHING*

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Dean and Professor of Microbiology
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Veterinary diagnostic laboratories may be physically located in one of the three following situations: where there is no veterinary academic institution, where a Department of Veterinary Science or Animal Pathology (School of Agriculture) is present, or where a College of Veterinary Medicine is located. Each environment can have a significant role in the teaching-research impact on animal agriculture of the community.

The laboratory, which is not related to academic veterinary medicine, has a distinct and important contribution to make in these fields:

a) Continuing education should be provided for practicing veterinarians in the region of state. Workshops and conferences, directed toward indigenous diseases or newly recognized or defined animal maladies, are needed. Information relating to field diagnostic techniques, utilization of laboratory services and cooperative field investigations can benefit the livestock owner, his veterinarian, and the control-regulatory programs of a given state.

b) Residency or Intern programs in veterinary diagnostics are needed. The non-academic associated laboratory may well provide valuable training in specialized areas unique to its given region or environment.

c) Research of an applied, as well as basic, nature has been most important in the past and has been conducted by astute observers in non-academic laboratories. Well trained diagnosticians will make contributions to our knowledge—regardless of their physical location. All veterinary diagnosticians should be encouraged to conduct applied research relating to the animal disease problems of the area.

The laboratory associated with, or in the proximity of, a Department of Veterinary Science or Animal Pathology can further expand its service mission:

a) Teaching of undergraduates in Animal Science, Poultry Science (in the School of Agriculture), etc., is a needed mission of the laboratory. The animal scientist should be aware of disease etiology, species transmissibility, control and hopefully eradication methods. The Federal-State programs on bovine tuberculosis, brucellosis and hog cholera, etc., should be logical topics for discussion and understanding. Public health aspects, or zoonotic implications, of animal diseases are important to all involved in animal agriculture.

b) Graduate Student education can be made a significant part of a cooperative program with a Department of Veterinary Science. Pathology, microbiology, physiology, toxicology, etc., may all be studied at the graduate level in the diagnostic laboratory and provide well trained diagnosticians.

c) Internships or residencies, research, and continuing education again are

*Presented at the 12th Annual Meeting of the Amer. Assoc. of Veterinary Laboratory Diagnosticians, Milwaukee, Wisconsin, October 13, 1969.
areas of needed and inherent activity which are a part of every diagnostic laboratory.

The diagnostic facility in physical proximity to a school or college of veterinary medicine offers some additional prospects for cooperative endeavor:

a) *Instruction of undergraduate veterinary medical students* provides a unique challenge and intellectual reward to the diagnostician. The classic duty necropsy stations for seniors are well utilized by most colleges. Critiques on necropsy cases combined with discussions of the clinical aspects of the case offer teaching value. Students can be stimulated to consider careers in diagnostic veterinary medicine through being a part of "small research projects" in the laboratory. In-depth laboratory instruction can further direct specific student interest in diagnostic pathology, microbiology, toxicology, etc.

b) As in all veterinary laboratories the opportunities for graduate study, internships, research and continuing education are paramount with the diagnostic facility associated physically with a college of veterinary medicine.

New areas or expanding concepts offer great impact in service, teaching, graduate study, and continuing education for a diagnostic laboratory involvement:

a) environmental health, i.e., water, air and land pollution monitoring and control.

b) Quality control — as well as the classical sanitation or hygiene approach in meat, poultry, dairy products and seafood examination.

c) toxicologic monitoring, particularly of chemical poisons as well as the phytotoxins.

d) diagnostics of exotic pet and zoo animals, e.g., fish, turtles, snakes, caged mammals and birds.

e) diagnostics relating to laboratory animals, e.g., rodents, carnivores, and primates. Services might be offered to the university community or expanded to include producers, industrial firms, or any agency or group within the state.

f) greater emphasis on companion animals, i.e., horses, dogs, and cats.

 g) wildlife disease problems and matters involving conservation.

h) establishment of national and international disease reporting and evaluation services.

The challenges facing the veterinary diagnostician and his laboratory are many and varied. No one laboratory can cover them all adequately. Much can be accomplished through educational programs for diagnosticians sponsored by a state or several state laboratories working in consort. Federal or regional diagnostic laboratories have an important role in the total educational mission as well. A well organized, coordinated and directed national educational program would appear to be in order. The AAVLD would be a logical organization to promulgate, sponsor, and initiate such a program to the benefit of all concerned.
 Anyone who has been intimately acquainted with the development and diversification of the veterinary profession during the past recent decades must marvel at the expansion and increased competence of its members. It is positively amazing, the changes that have taken place, and this varies among all phases — whether you are in practice, research, education, regulatory, extension, commercial, professional organization, military, international, public health, etc.

Growth can come just from numbers of new graduates, but increased competency has come not only from better primary education but most important from some type of continuous education following graduation from our veterinary colleges. This type of education takes all kinds of forms, from formal graduate work to attendance at meetings, such as this, or on-the-job training, etc.

The profession didn't become extinct when they phased out the working horse in this country as some had forecast; instead, it adjusted accordingly and today we see it flourishing in many different areas of endeavor. There was a time, though, when experience alone after graduation was thought by many to be the only effective process of continuing education. This is recognized today to be obsolete thinking. A profession can become obsolete quickly if it doesn't recognize the need to keep current. This is no easy task because of the accelerated rate of new technical knowledge that seems to become available daily. I would like to emphasize the rate of change because we have always lived in a changing environment.

Speaking of a changing environment, look at what has happened to agriculture in recent decades. In 1945 we were raising livestock on 4,600,000 premises. In 1965 we raised over 9 percent more livestock than we did in 1945, and on 2,300,000 premises. It has been forecast that by 1980 we will be raising more livestock in this country on 1,000,000 premises.

None of us are startled when we say this has had an effect on our profession. Any large animal practitioner, or ex-large animal practitioner, will let you know the effect that it has had on them. Not all bad, either. Some have a much better net income and less arduous work in small animal medicine, or other branches of the profession, while others have been very successful when they remained in large animal practice.

In the area of regulatory medicine, with which I have been closely associated, we have seen dramatic changes in both the Animal Health and Veterinary Biologics Division activities — all warranting much greater professional skills. All recognizing that some type of continuous education is necessary — in fact, essential.

All of us recognize and appreciate the constant reminders by our clinic or veterinary medicine professors that anyone could treat an animal effectively if he had the ability to diagnose accurately. This is as true today as it has been in the
All we have to do today is step into one of our diagnostic laboratories to realize the changes that have occurred in that part of the profession. It is as true as it has always been that they will vary in their capabilities, but as a whole, the changes compared with a few decades ago are quite obvious.

However, as one of your colleagues said to me, "The reason why the changes are so obvious is that we have had too far to come. The cost of equipment is so high and the equipment becomes obsolete so quickly that we give our procurement officers nightmares. We are still a long way from keeping abreast of the newest developments in the majority of our diagnostic laboratories.

Of even greater concern to us is the need to develop people to meet the demands upon our laboratory. We can't hire good people at our salary levels and when we train and develop them, we lose them. "It's a real dilemma."

Despite this bleak outlook we know that some laboratories have been able to overcome these obstacles. It doesn't mean that they have overcome the problem. It means they have been able to adjust to it — and not necessarily by accepting lower quality work.

The demands of practitioners, regulatory programs and industry being served by these laboratories have been increasing at a great rate, as all of you know. I don't see this diminishing.

Many laboratories serving veterinarians who are on retainer fees are demanding results for all kinds of tests as part of their preventive medicine portion of their practice. They are more concerned with the total herd problem than the need to treat individual animals. This is in contrast to the old days when the veterinarian was being called out to diagnose and treat animals after the owner had tried everything else.

In our areas I see us demanding better diagnostic services. I see us weighing the losses caused by those diseases that have the greatest economic significance and programs being developed to reduce them. I see you becoming more deeply involved in diagnosing food-borne diseases such as salmonellosis, aflatoxins, microtoxins, drug antibiotics and pesticide residues. Also, zoo animals and wildlife are increasing in great numbers which will also present some interesting disease diagnostic challenges.

One young enterprising man from California told me that he was expecting to collect a blood sample from an animal, insert it into a computer and be able to read results of all the diseases that the animal may have had which produced changes in its blood. We may not be quite that sophisticated, but it is not impossible as we look to the future.

Several years ago, as I recall, we took an inventory of each laboratory's disease diagnostic capabilities. The purpose was to determine the various diagnostic services available throughout the country. This not only showed us what capabilities were available, but also what was not available. Even in those days it was quite impressive.

The purpose of this paper is to put forth a challenge to see whether everything is being done, not only to acquire and maintain the best equipment, but also the most competent people in our disease diagnostic laboratories.

I'm sure that when it comes down to a final evaluation of the effectiveness and
efficiency of a laboratory it takes both good equipment and good qualified people. The latter — in most cases — can’t be bought. They must be developed.

This, again, is not always bad. Recently, I was on a committee that interviewed college graduates who had been working for the Department for just a few years. We found the best morale among those who had a feeling that they were developing on the job. In fact, those who were discontent were not complaining about salaries but lack of job satisfaction.

It was interesting to note, also, that those who were enthusiastic about their jobs worked for supervisors who were also enthusiastic about their jobs. Their supervisors showed keen interest in their development.

Again, where we observed great discontent among them, those employees were brought into the organization and, for all practical purposes, were being ignored. They were left to sink, or swim.

Some folks have said to me “What is so bad about that? It happened to me when I came aboard, and I learned to swim damn well and fast!” That’s great for those who have this ability, but there are some who never will swim because no one helped them, and others who couldn’t swim no matter how much help they got. What I’m stressing is that the latter two groups drag our organization down and we should not only train and develop the natural swimmers, but also the ones who can learn if given a chance. As soon as we can recognize the permanent non-swimmers and get them out of the organization, the better.

I believe, though, that training and development is not a hit or miss proposition. It must be well thought out, well planned. People in charge of laboratories need to have a good unbiased evaluation of their personnel. They need to know their personnel and give them frequent counseling — counseling not only in reference to how well they are doing on the job, but counseling on their future.

One authority said that since he left the bench he has become a motivator. His main task is not how to motivate others, but how to get them to motivate themselves.

Growth and achievement appear to be the two basic factors found in job satisfaction. They also seem to go hand-in-hand as one grows in his job and achieves more, he is motivated to grow more.

If we accept the fact that we are living in the environment with an accelerated rate of change, then this growth is essential to meet the needs of our times. We also recognize the need for some type of development program throughout our careers.

Most of us have not been fortunate enough to have had good supervisors that seemed to appreciate this need and took the time and effort to help us develop a growth plan. Many of us complain that what we need are better qualified veterinarians to come to work for us, and that they should have gotten this type of training during their work at the veterinary colleges.

I serve on a Committee in the Department of Agriculture which is made up of USDA administrators and Land-Grant College officials. The purpose of the Committee is to exchange ideas on the type of employee we would like to see come out of our universities 10 or 20 years from now and work for us. Every specialty group that appears before them asks that the curriculum be changed and more emphasis be placed on their specialty.

Realistically, this isn’t the answer. The fields are expanding so that each
specialty must recognize its responsibility of developing their people. We all need basic backgrounds, but refinements of specialty is something else.

In fact, laboratories must rely on one another for training and development of laboratory personnel. There were times in the past when discussions with some of our educators on our needs were falling on deaf ears. They couldn't quite grasp what was needed. However, by getting certain staff in universities involved in our work we finally got the type of training we needed, as well as good counseling from them on our personnel development plan.

As usual, our needs were best filled by a variety of approaches. Meetings, seminars, short courses, transfers between stations, exchange of personnel, on-the-job training, graduate work, etc. The key to development was counseling and good evaluation. This enabled us to train those from whom we expected to get the best results.

We also found out that there are those who are loud talkers about training and development, but who really aren't committed to it. A good indication was when funds were cut, the first item those individuals chopped off was training and development.

Besides training veterinary personnel there is also a demand to train support personnel. In fact, one chief complaint raised by a person evaluating laboratory activities was that quite often scientists or professionals, are spending too much time doing what we would call "support work." So, as we develop the professional there is a constant need for similar types of programs for support personnel.

Finally, there is another vital factor always associated with job satisfaction. And that is recognition. Those who work hard at their personal growth and development should be recognized. It appears that with the sophistication that has been developing in your laboratories, it is time to have some type of accreditation, or recognition, both of the laboratories and of those who work within them.

I know there are boards within some specialties, but I'm thinking of a broader type of recognition. This would allow those among you to set lofty goals and seek to achieve them.

It might help you to get better equipment and better people if you were able to show that other laboratories had better recognition than yours. New employees would want to work in laboratories with that type of recognition or for workers who had received recognition from those in your veterinary diagnostic laboratory association. What better evaluation and recognition can one get then to be rated by those who are working within your specialty.

I'm sure that most of these points have been considered by you many times. I have seen so much good come from the training and development of personnel within an organization that I welcome this opportunity to share my thoughts with you. I don't believe there is anything more important for a person who is supervising people than to give them an environment in which they can grow and develop, and society can reap the rewards of these talents.

I congratulate you on the advances you have made. All of us who are deeply dependent on your services know that you will meet the demands of the future even better than you have in the past.
DIAGNOSTIC LABORATORY GRADUATE TRAINING AND CONTINUING EDUCATION

Dr. V. A. Seaton
Ames, Iowa

The animal industries of the United States have always been important in the past and so long as food of animal origin remains an important part of human food consumption, the food industries will continue to be of major interest in the future. I consider the profession of veterinary medicine and its activities as a profession to be significant in food production. This then indicates that regardless of the means by which we make our contributions in veterinary medicine, we will have to make our individual efforts count for more and be more productive as our population grows. It also indicates that regardless of however inefficient or inadequate we may have permitted ourselves to be in the past, we will not be able to afford that luxury in the future.

The diagnostic laboratory role in the overall scheme of things in our profession is not only that of diagnosing animal diseases, but is also one of monitoring what is going on. We ask these questions. What are the disease trends? What diseases are we now seeing by new manifestations due to changing management conditions? How significant is a particular disease, nationwide? Is a specific disease a greater problem in another area of the country than in our own? Or less so? What about transportation nationally and internationally, of heretofore, unrecognized diseases in our country? What about environmental changes, which we are all being subjected to? What non-infectious conditions are occurring in our animal population which must be diagnosed and for which an appraisal must be made of their significance? What are the trends?

We must be broad in outlook, and concerned beyond our state lines. This points up disease reporting, for instance, from diagnostic laboratories but even more pertinent to this panel discussion today, it points up our need for competence and how we acquire it, if we do not have it.

I am happy to participate in the discussion as it affords an opportunity to discuss:

1) What is diagnostic medicine? and,
2) Means of training diagnostic personnel.

Diagnostic laboratories afford an opportunity to see and to participate in veterinary medicine in a unique way and from a point of view that is afforded no others in the profession. It represents the epitomy of the effort to bring the knowledge of the basic and clinical sciences to bear on a specific herd or flock problem. It is in the diagnostic laboratory that anatomy, physiology, microbiology, pathology, pharmacology, toxicology, and the clinical sciences, including epidemiology, are correlated with the laboratory findings, and finally, that meaningful and productive interpretations are made. THAT IS DIAGNOSTIC MEDICINE.

The need for competence cannot be overemphasized in diagnostic medicine. There is no substitute for competence.

Exposure to diagnostic medicine must begin in the undergraduate or
professional curriculum. This should be provided by actual participation on the part of the students in diagnostic laboratories wherever available. Some colleges of veterinary medicine are doing this now. The student sees an aspect of his professional life in the diagnostic laboratory that is not available to him elsewhere.

Too often in our undergraduate curriculum, we are departmentalized in our learning to the point of never being able to bring the sciences together in a meaningful and comprehensive way until we are suddenly faced with professional problems which demand comprehensive views. I fear we often fall short at that time, or at least our learning at that time, is under more difficult circumstances than need be. This is why undergraduate exposure to diagnostic laboratories is so important. For it is here that experience in making correlations must begin.

Specialization in our entire profession is with us now it is especially true in diagnostic laboratories. We must have trained personnel in the various disciplines. Specialists must lead the way in advancing our knowledge and applying it to use in diagnostic medicine.

In my opinion, there is no one way to train people for diagnostic medicine. Certainly experience is a great teacher, but so are graduate programs and so are training programs for specialty boards. I will mention a graduate program in a little more detail later.

I would like, at this point, to make brief mention of research activities in a diagnostic laboratory. My only point to be made is simply this. Research and diagnostic services are compatible. One feeds the other and a facility with either research or service alone can soon become sterile.

Many conflicts in the past between diagnostic service and research in a single laboratory, and even some presently existing over the country today, are the result, in my opinion, of improper budgeting and administration — not the results of a basic incompatibility between research and diagnostic services.

One training method I have been asked to discuss in connection with our panel is the non-thesis master’s program.

The purpose of the M.S. degree is to provide breadth as well as depth, but is simply without emphasis on a major research problem. This is compensated for by special topics and smaller research projects in various diagnostic disciplines and by actually working in the diagnostic laboratory with specialists in their respective fields. This affords to my way of thinking, a potentially strong masters program because the course work is the same as in basic graduate courses already existing but the emphasis is in multiple disciplines such as pathology, bacteriology, virology, immunology, toxicology, and chemistry, rather than majoring in only one. The other strong advantage in working in the laboratory on diagnostic cases and problems with persons who have advanced training or board certification in their respective discipline. The course work would be variable depending on specific needs and the interest of the candidate. The length of the program would be six or seven quarters or a total of some 18-20 calendar months.

Certain core courses would exist in all such programs, but the individual would have maximum flexibility in the remainder of his program.

A guideline for one interested in diagnostic work might include courses cellular pathology, physiological chemistry, advanced postmortem techniques, histopathological slide seminars, veterinary toxicology, neuropathology, serology, immunolo-
gy, virology, bacteriology, and special topics in any one of the above areas.

The program would generally range from 12-14 credits per quarter. Examinations associated with this program include:

1) an 8-hour practical examination utilizing histopathological slides, kodalines and other testing procedures.
2) an 8-hour written examination.
3) an oral examination by the graduate student's committee.

This program at Iowa State University is of recent origin. Doctor Frank Ramsey, one of the fathers of the program and Head of the Pathology Department, is the man responsible for this training program. If anyone should be interested in discussing a non-thesis master's program in diagnostic medicine, I suggest he contact Doctor Ramsey for the specifics of program which might fit the needs of that particular person. The program is flexible and I believe it may be yet another way in which we may speed the competence to greater numbers of people in diagnostic medicine.

Continuing Education in Diagnostic Medicine in Iowa

This program is coordinated by the Iowa Academy of Veterinary Practice which is a subsidiary of the Iowa Veterinary Medical Association. The purpose of the academy is to raise standards of both practice and of the services available to practitioner's clients. One of the main purposes, however, is to encourage academy members to participate in different kinds of continuing education programs.

They must reach a required number of credits per year to maintain their membership in the academy. Our diagnostic laboratory now has setup a voluntary program as follows:

The practitioners spend three days together in our laboratory. They are scheduled far in advance. The schedule is rather rigidly followed. Our purpose is to acquaint them with the philosophy of our laboratories, policies of the laboratory and to discuss diagnostic laboratory approaches to their field problems.

We provide them with various instruction sheets on collection, preservation, and submission of specimens for bacteriology, virology, and toxicology. Each of the three mornings is spent with the practitioners being oriented to the laboratory, discussing procedures and techniques employed in the laboratory so they learn what to submit and what to expect in return. Considerable time is spent on interpretation of our laboratory results and their correlation with the practitioner's findings in the field. The three afternoons are spent with the pathologists in the postmortem room — actually participating in the handling of cases. The program is most successful from our point of view and judging from their written and oral critique at the end of the three-day session, it is of great benefit to them. I believe it is a most useful continuing education program. I would encourage other diagnostic laboratories to initiate such programs, because I believe if practitioners are brought up to date on laboratory capabilities, they will use laboratories more intelligently and the laboratory personnel's time will be better spent.

Briefly, I have presented an outline on a non-thesis master's degree for training of diagnostic personnel and also outlined a current continuing education program in our laboratory in Iowa.

All such efforts are a part of what we are talking about today and the mere
presence of this panel on this program seems to me to signal that the time has come for greater efforts in laboratory training.

I believe competency in diagnostic laboratories is so important, because the effectiveness of the veterinary profession in the future will be no better than the effectiveness of diagnostic laboratories. To paraphrase a well-known saying, but apply it to Veterinary Medicine – "Once Diagnostic Medicine Begins, All Other Arts Follow", in the veterinary medical profession.

THANK YOU.
SEROLOGIC SURVEY OF TOXOPLASMOSIS IN SOUTHERN CALIFORNIA DOMESTIC ANIMALS BY A HEMAGGLUTINATION, MICROTITER METHOD

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Introduction

Toxoplasma gondii is a parasitic protozoan which infects a wide range of natural hosts including man.

A study by Jones et al (11, 12) revealed a titer for Toxoplasma gondii in 25% of 3,796 women in the childbearing age in the Los Angeles Metropolitan Area. A significantly greater incidence of abortion occurred in patients whose post partum blood sera had a high antibody titer for toxoplasmosis than in those with low or negative serum levels. This survey revealed three women who habitually aborted for unknown causes out of 89 women with titers of 1,024 or more and at least nine other subjects in the high antibody titer group who had pregnancy histories suggesting the possible relation of the parasite to abnormal pregnancy outcome. Review of total pregnancy history of these high titered patients has identified a series of infants for follow-up investigation by these workers for subclinical congenital toxoplasmosis.

Serum samples from 1,204 persons were tested for reactivity by the hemagglutination and methylene blue dye tests for toxoplasmosis (Lewis 16, 17). A smaller number were tested for complement fixing antibody.

Since it was possible to demonstrate the parasite in only two of the persons, most of the sera came from persons who were suspect but not proved to have clinical toxoplasmosis.

The highest prevalence rates of hemagglutinating antibody were found in persons: (1) with chorioretinitis that, from the clinical history, was thought to be noncongenital; (2) with congenital chorioretinitis; (3) who were family contacts of suspected cases; and (4) with neurologic problems.

Intermediate prevalence rates of antibody were obtained from persons with: (1) lymphadenopathy; (3) hepatosplenomegaly or jaundice; and (3) anterior uveitis.

Prevalence rates were lowest in: (1) persons with miscellaneous diseases, e.g. pneumonitis, skin rash, etc.; (2) persons with fevers of undetermined origin; (3) medical students and laboratory workers; and (4) "normal" residents of Southern California.

Persons with chorioretinitis that was thought to be noncongenital comprised the largest clinical group. Their serologic results differed statistically from those of persons in the "normal" group in two respects: (1) they exhibited the highest prevalence rate of hemagglutinating antibody of all the groups; and (2) although the distribution of titers in the complement fixation and methylene blue dye tests did not differ statistically from results in the "normal" control group, the
titers in the hemagglutination test were significantly higher than those of the controls. These differences were of sufficient magnitude that they may aid in distinguishing between persons with chorioretinitis caused by *Toxoplasma* and persons with asymptomatic toxoplasmosis and concurrent, but unrelated, chorioretinitis.

Serologic tests for toxoplasmosis exhibited remarkably stable titers that did not decrease significantly in response to adequate therapy.

Dr. McCulloch (18) in his fine paper presented before the American Association of Veterinary Laboratory Diagnosticians last year, urged veterinary diagnostic laboratories to use and evaluate diagnostic tests presently available for toxoplasmosis. A survey was undertaken by the Los Angeles County Veterinarian to provide comparative information regarding the prevalence of subclinical infection in domestic animals in the Los Angeles area and to study the feasibility of the hemagglutination test for use in our laboratory. The technique described in this paper is that of Jacobs and Lunde and as modified by Lewis and Kessel (16, 17). We have further adapted these methods to the microtiter method.

Various means of serologic testing have been explored since the *in vitro* dye test of Sabin and Feldman was described in 1948. One of the chief criticisms to the dye test is that it requires live parasites which are a realistic biological hazard. Secondly, there is a greater variability in the standardization of the antigen when using live organisms.

Another procedure based on hemagglutination methods outlined by Boyden (1951) and Stavitsky (1954) was reported by Jacobs and Lunde (1957) for detecting antibodies of toxoplasma using tanned sheep cells to which *Toxoplasma*, antigen was adsorbed. As an antigen, they utilized an aqueous extract of *Toxoplasma* organisms which was obtained from the peritoneal fluid of infected mice.

Blood samples for our survey were obtained from 152 swine on Los Angeles County hog ranches; from 476 dogs, mostly from Los Angeles County Animal Shelters; from 152 cats—pound cats and sera submitted to our laboratory; from 94 goats presented for brucellosis testing; from 83 monkeys—mostly rhesus—at research institutions; from 218 horses, 36 purebred and 182 privately-owned pleasure horses; and 134 cattle presented for brucellosis testing.

**Materials and Methods**

I. *Toxoplasma* Antigen Preparation

A. Inoculation

1. Caution should be exercised in working with live *Toxoplasma* trophozoites.

2. The original inoculum of the Rh strain of *Toxoplasma gondii* was obtained from the Doheny Eye Foundation, Los Angeles. This and all subsequent inoculations of 0.5 ml. were given intraperitoneally with a 22 ga. needle. The inoculum was made up in aliquots of 5 ml. This was prepared by using 5 ml. of pH 7.2 buffered saline plus peritoneal exudate (averaging 0.5 ml.) from one or more inoculated mice. Peritoneal exudate was considered to be satisfactory when a
count of 30-35 viable trophozoites were found per X400 microscope field.

3. After receiving intraperitoneal inoculations with living trophozoites, mice succumbed in three to five days. It was important to start the peritoneal harvest within a few hours after the first mice started to die. This was usually on the third day after receiving the peritoneal exudate.

B. Harvesting of Antigen

An ice bath was prepared using a 500 ml. beaker. Two or more 15 ml. screw-capped tubes containing 4.5-5.0 mls. of pH 7.2 phosphate buffer were then placed into the bath.

Mice were euthanized in a bell jar. The peritoneal fluid was slowly withdrawn from each animal using a Leur-Lok 2 ml. syringes with an 18 ga. needle. Fluid grossly contaminated with blood was discarded. After filling a syringe, the fluid was then carefully emptied below the surface of the cold buffer solution in the screw-capped tube. This process was continued until a total of about 6-7 mls. of peritoneal fluid was harvested and added to each screw-capped tube and all animals were sacrificed.

An hour after beginning harvest, the vials were centrifuged at 1500 RPM in a refrigerated centrifuge for 5-10 minutes. The supernatant was poured off and the sediment was resuspended with the cold buffer solution. The washing procedure was repeated 3-4 times after which the supernatant was poured off and the cells held in the refrigerator above freezing.

C. Extraction of Antigen

This involved the removal of soluble fractions of antigen from Toxoplasma trophozoites. Extraction was based on the addition of cold distilled water which lysed cells and thereby killed viable organisms and released the antigen. It was found best to add the minimum of cold distilled water (one part cells to ten parts water) to prevent “overdiluting” of the antigen. After 30 minutes, a wet smear of the suspension was made to see if more distilled water was necessary to lyse all cells.

The suspension was then held in the refrigerator for 30-60 minutes. Then an equal quantity of 1.7% NaCl was added to the suspension of lysed cells. This suspension was then put into 50 ml. plastic centrifuge tubes and spun at 16,000 RPM for 30 minutes in a refrigerated centrifuge. Later, the supernatant was saved and the sediment discarded.

D. Standardization of Antigen

Four dilutions of our final antigen was tested against the Sabin-Feldman titer of human sera from the Doheny Eye Foundation. The microhemagglutination test method was used.
At this point we decided that, since nine of the ten samples agreed within one dilution at the one-ten dilution, we would continue with that dilution of our antigen.

E. Micro-Hemagglutination Test

1. Stock Solutions (made from deionized water and stored in refrigerator; all glassware should be acid cleaned):

   a. 0.15 M KH$_2$PO$_4$ (isotonic) 9.07 g./l. saline
   b. 0.15 M Na$_2$HPO$_4$ (isotonic) 9.50 g./l. saline
   c. 0.85% NaCl
   d. 1:1000 tannic acid

2. Working Solutions:

   a. pH 6.4 Phosphate Buffer (6.4 - PB)
      Prepare with approximately two parts 0.15 M KH$_2$PO$_4$ to one part 0.15 M Na$_2$HPO$_4$ (adjust this buffer, as well as the pH 7.2 buffer, to the correct pH using only the stock solutions -- do not use acid or base to adjust pH.)
   b. pH 7.2 Phosphate Buffer (7.2 - PB)
      Prepare with approximately one part 0.15 M KH$_2$PO$_4$ to two
parts 0.15 M \( \text{Na}_2\text{H PO}_4 \).

c. Normal Saline and pH 7.2 Buffer (PBS – 7.2)
Prepare by adding one part 7.2 buffer to three parts 0.85% \( \text{NaCl} \).

3. Procedure (all steps are performed with cold solutions, except for attachment of antigen to RBCs – Step e.):

a. Blood Collection
Collect a minimum of 3 ml. of whole sheep blood in EDTA. Mix one part of ACD preservative with four parts blood in a clean tube; refrigerate. Date blood tubes and do not use blood more than ten days old. Goat RBCs were found to produce more consistent results with swine serum.

b. Washing of Red Blood Cells
Cells must be washed four times with PBS – 7.2 in the following manner. A tube of whole blood prepared as above is centrifuged 3-4 minutes at 2000 RPM. Draw off the supernatant. Break up the packed cells in the bottom of the tube by flicking with fingers. Add about ten volumes of cold PBS – 7.2 to one volume of cells, mix well and centrifuge as above. This procedure is repeated for a total of four washes. At this point, prepare a 37°C water bath.

c. Determination of Volume of RBCs
(1) After the fourth wash and spin, break up the button of packed cells and add 5 ml. of PBS – 7.2 to cells in tube. Suspend cells well and pour into a calibrated centrifuge tube and bring the volume to 10 ml. with PBS – 7.2. Centrifuge for five minutes at 2500 RPM. Read volume of RBC.

(2) Tanning requires a 3% solution of RBCs. To make this 3% solution, add 32 volumes of PBS – 7.2 to one volume of RBCs.

(3) 2 ml. of this 3% solution is added to each of as many tubes as are required for antigen and control cells. Refrigerate these tubes.

(If 1 ml. of antigen – which is sufficient to run 80 tests – is being used, it will be diluted to 9 ml. so a total of nine tubes, each containing 2 ml. of 3% sheep cells, will be prepared.)

(4) Prepare an ice bath.

d. Tanning the RBCs
(1) A new stock solution of tannic acid should be prepared for each new batch of sheep RBCs at least every 14 days.

0.1 gm. tannic acid in 100 ml. distilled water = 1:1000 stock tannic acid solution. To prepare the working solution of 1:100,000 tannic acid, dilute the stock solution one to 100 with PBS – 7.2.

(2) If nine tubes of RBC suspension have been prepared as above, then 20 ml. of 1:100,000 working tannic acid
solution is sufficient. This is prepared by adding 0.2 ml. of
1:1000 stock tannic acid solution to 19.8 ml. of
PBS – 7.2.

(3) Resuspend the RBC in the nine tubes before the 1:100,000
working tannic acid solution is added. When cells are in
suspension and ice bath is ready, 2 ml. of 1:100,000
working tannic acid solution is added to each of the nine
tubes.

(4) Stopper tubes, invert once to mix, and place in ice bath for
15 minutes. While in the ice bath, invert at five-minute
intervals to resuspend cells.

(5) Centrifuge – three minutes at 200 RPM. Draw off the
supernatant.

(6) Break up button in tubes – resuspend in PBS – 7.2 and
pour into clean tubes. Centrifuge once more – three min-
utes at 2000 RPM. Draw off supernatant.

(7) Break up button in tubes. Resuspend in 2 ml. of 0.85%
NaCl. Transfer from tube nine 1 ml. of tanned RBC in
0.85% NaCl to a tenth tube. There are now eight tubes
containing 2 ml. of the tanned RBC in 0.85% saline and two
tubes each containing 1 ml. of this suspension. The cells at
this point are very sensitive and ready to accept the
antigen. Refrigerate.

Label four tubes containing 2 ml. and one tube
containing 1 ml. of above suspension as controls and the
other five tubes (four 2 ml. and one 1 ml.) as test cells.
Mark 1 ml. tubes clearly.

e. Attachment of Antigen

(1) Thaw a tube of antigen (1 ml. QS for 80 serum samples).

(2) When using sheep RBCs with present antigen, dilute 1 ml.
antigen to a total volume of 9 ml. with cole 6.4 – PB.

(3) Add one volume of the antigen diluted with cold 6.4 – PS
to one volume of the tanned sheep RBCs diluted with
normal saline in the five tubes marked test. Do not add
antigen to control tubes.

(4) Stopper tubes, invert once, and incubate in a 37°C water
bath for 15 minutes. Invert the tubes at five-minute
intervals.

(5) Centrifuge for three minutes at 2000 RPM. (This includes
all ten tubes – test and controls, as does the next step.)

f. Final Wash of Cell with Stabilizing Serum

(1) Rabbit serum in 2 ml. quantities is stocked in Revco at
-82°F.

(2) Dilute rabbit serum to 2% (2 ml. rabbit serum in 98 ml.
cold PBS – 7.2).

(3) Break up button in tubes and resuspend in at least 10 X
cell volume of stabilizing serum.

(4) Centrifuge tubes for three minutes at 2000 RPM. Draw off
SEROLOGIC SURVEY OF TOXOPLASMOSIS IN

the supernatant and repeat once.

(5) Break up button in tubes and resuspend with stabilizing serum to total volume of 3 ml. in the eight tubes containing 2 ml. of tanned cells in saline and 1.5 ml. in the two tubes containing 1 ml. of tanned cells in saline.

(6) Pool the five control tubes in one large tube and the same for the five tubes of test cells. Label clearly.

(7) Refrigerate until time to run tests.

g. Testing Serum

Set up plate as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0 0 0</td>
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<tr>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0 0 0</td>
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<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

2 8 32 128 256 512
(Reciprocals of serum dilution in each tube.)

(1) Divide the plate in half as illustrated by line A. Mark rows 2, 3, 4, 8, 9, 10 across as shown in red at B. The wells in these six rows down are to receive three drops each (.075 ml.) of saline. The other six unmarked rows are to receive one drop (.025 ml.) saline in each well. Serum diluted with saline in this manner results in the dilutions marked below the sketch of the plate. Label right half of the plate 'control' and left half 'test'. Mark sample numbers on edge of the plate.

(2) Diluting serum. Flame microdiluters to glowing before use and after each serum is titered. Dip a diluter in the first sample, twist several times, and place in the first well on test side of plate designated for that sample. Leave diluter in well and pick up next three samples in the same manner. Hold all four diluters between fingers, twist in well 10-15 times in first row of wells. Pick up all four diluters together and place in row 2. Continue this procedure through row 6 (to line A). Repeat this dilution with the same four samples on the control side of plate. Four more sera may be tested then on lower half of the plate.
(3) Addition of cell suspensions. Use microdropper to add to each well on test side of plate, one drop (.025 ml.) of test cell suspension (cells with antigen attached). To each well on control side of plate add one drop of control cell suspension. Gently agitate plate of cells to insure good suspension of cells before filling microdropper.

Seal plate on the tape dispenser. Shake plate gently to mix. Note time, leave undisturbed, and read in one hour.

(4) Reading titers. The titer of the serum being tested is read as the dilution in the tube preceding the first well in which the cell button appears to be of uniform size with the buttons in the following wells. The controls are read in the same manner and the actual titer of the serum, if the control is not negative, is determined by dividing the test titer by the control titer. If the titer appears to be beyond the sixth well (1:512), then the serum must be retested using one full row of twelve wells. When this is done, three drops (.075 ml.) saline are placed only in rows 2, 3, and 4 and one drop (.025 ml.) in wells 1 and 5 through 12.

Results
Our results are similar to those reported by McCulloch et al (19) in their survey of toxoplasmosis in Iowa domestic animals. We reported titers at all dilutions. The significance of the lower titers, particularly at a 1:8 dilution and lower, is undetermined at this point. Our percentages of animals showing any titer would be significantly lower had we ignored the 1:2, 1:4 and 1:8 dilutions. It was not feasible to collect paired samples in our survey.

As with most serological studies, antibody measurement represents only a mirror image of a disease and certainly is not a completely accurate indicator insofar as disease incidence is concerned.

We have diagnosed very few cases of toxoplasmosis in individual animals in our laboratory by histological, cultural or other means.

Summary
A microtiter hemagglutination technique for toxoplasmosis is described. There were 1,309 serum samples collected from dogs, cats, monkeys, goats, swine, cattle, and horses in the Los Angeles area. Results of these tests as expressed in percentages showing titers 1:2 or higher are as follows: 476 dogs – 20%; 152 cats – 18%; 83 monkeys – 12%; 94 goats – 60%; 152 swine – 74%, 134 cattle – 1%; and 218 horses – 21%.
<table>
<thead>
<tr>
<th>Species</th>
<th>Number Tested</th>
<th>Negative</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
<th>1:512</th>
<th>1:32,000</th>
<th>% Having</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procine</td>
<td>152</td>
<td>39</td>
<td>-</td>
<td>-</td>
<td>23</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>59</td>
<td>1</td>
<td>-</td>
<td>74%</td>
</tr>
<tr>
<td>Canine</td>
<td>476</td>
<td>382</td>
<td>21</td>
<td>39</td>
<td>12</td>
<td>16</td>
<td>1</td>
<td>5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>20%</td>
</tr>
<tr>
<td>Feline</td>
<td>152</td>
<td>114</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>--</td>
<td>1</td>
<td>1</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Caprine</td>
<td>94</td>
<td>37</td>
<td>13</td>
<td>4</td>
<td>15</td>
<td>12</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>--</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>Primates</td>
<td>83</td>
<td>73</td>
<td>9</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12%</td>
</tr>
<tr>
<td>Equine</td>
<td>218</td>
<td>170</td>
<td>22</td>
<td>-</td>
<td>20</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21%</td>
</tr>
<tr>
<td>Bovine</td>
<td>134</td>
<td>128</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1%(0.4%)</td>
</tr>
<tr>
<td>TOTALS</td>
<td>1309</td>
<td>943</td>
<td>36</td>
<td>44</td>
<td>94</td>
<td>35</td>
<td>67</td>
<td>3</td>
<td>83</td>
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<td>27%</td>
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</tbody>
</table>
BIBLIOGRAPHY


THE LABORATORY DIAGNOSIS
OF BOVINE VIRUS DIARRHEA
BY FLUORESCENT ANTIBODY

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INTRODUCTION

The existence of noncytopathic strains of BVD virus presents a special problem to the diagnostic laboratory attempting to isolate this virus. In the past the detection of such noncytopathic strains depended upon the transmission of the disease to susceptible calves by inoculation of materials from infected animals, a procedure impractical for most laboratories testing large numbers of specimens. The indirect methods developed for detecting noncytopathic BVD strains in tissue culture, the 'cellular resistance' test\(^1\) and the 'END' test\(^2\), apparently have not been widely used in diagnostic work.

Fernelius et al\(^3\) showed that BVD virus could be visualized in tissue culture by immunofluorescence. The need for a practical sensitive diagnostic test to identify both cytopathic and noncytopathic strains of BVD virus in clinical and post mortem specimens prompted us to study the suitability of a fluorescent antibody tissue culture method for routine diagnostic purposes. We report here the details of this study and the results of applying the method to bovine specimens submitted to the Wisconsin Animal Health Laboratories for virus examinations during one year.

MATERIALS AND METHODS

A. PREPARATION OF HYPERIMMUNE SERUM:

The noncytopathic strain of BVD virus, New York 1 (received from Dr. J. H. Gillespie, Cornell University, Ithaca, New York), was propagated in secondary bovine kidney cells maintained with Hank's lactalbumin hydrolysate (HLA) medium with 2% fetal calf serum.** Infected culture fluids were collected after 3 days of incubation and concentrated 50-fold by ultracentrifugation at approximately 33,000 \(x\) \(G\) for 8-9 hours. A yearling calf was hyperimmunized by five intravenous injections of 5 ml of the concentrated antigen on consecutive days. One month later on successive days, two 5 ml booster doses were given and after 20 days the serum was collected. Prior to immunization, the calf had no neutralizing antibody to BVD, IBR, or PI-3 viruses. After immunization, the serum neutralization titer was 1:1280 against 100 TCID\(50\) per 0.1 ml Oregon C\(24V\) strain of BVD virus. No titers developed against IBR and PI-3 viruses.

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**Grand Island Biological Company, Grand Island, N. Y.
B. PREPARATION OF CONJUGATE:

The immune serum was first applied to a column of Sephadex G-25* previously equilibrated with 0.015M phosphate buffer, pH 6.3.\(^4\) Gamma globulin was separated from the serum by a chromatographic method employing DEAE-cellulose (Selectacel 20)**; elution was with the same phosphate buffer.\(^5\) The protein-containing fractions were concentrated using Aquacide II***. The preparation of gamma globulin was free from detectable impurities as judged by starch gel electrophoresis (done by Dr. O. Smithies, University of Wisconsin, Madison). Conjugation with fluorescein isothiocyanate**** followed essentially a method previously described\(^6\) using 0.025 mgm of isothiocyanate per mgm of protein. Unconjugated fluorescein isothiocyanate was removed from the preparation on a Sephadex G-25 column.\(^4\) The specific conjugate was absorbed twice with bovine kidney powder and stored in small aliquots at -70°C. At this temperature the conjugate has retained its staining capacity for more than one year. The original antiserum stored at -20°C is still providing material for making satisfactory conjugate after 2½ years.

C. FLUORESCENT ANTIBODY TISSUE CULTURE PROCEDURE:

Post mortem and clinical specimens are prepared as 10% suspensions in Hank's balanced salt solution (HBSS) containing 0.5% gelatin and antibiotics (penicillin, streptomycin, kanamycin and amphotericin B). The suspensions are centrifuged at 10°C for 20 minutes at 4300 x G, except for specimens from the intestinal tract which are centrifuged at approximately 9500 x G. The supernates are retained for testing.

Secondary bovine embryonic kidney cells (BEK) suspended in HLA with 15% fetal calf serum are grown on cover slips in Leighton tubes and are used in 2 to 3 days when monolayers are formed. After removal of the growth medium the monolayers are washed with 2 ml of HBSS for 30-45 minutes. The HBSS is removed and 0.1 ml of each supernate is inoculated into a Leighton tube culture. A drop or two of maintenance medium (HLA with 2% chicken serum) is added and the cultures are incubated at room temperature for 30 minutes to allow adsorption of the virus. 0.9 ml of maintenance medium is then added and the cultures are incubated for 4 days at 37°C.

The cover slips are washed with two changes of HBSS for 20-30 minutes, fixed in acetone for 10 minutes at room temperature, rinsed in 0.01 M phosphate buffered saline (PBS), pH 7.2, for 5 minutes and air-dried. The monolayers are covered with the specific conjugate, (diluted 1:5 in 0.01 M PBS, pH 7.8) and incubated in a moist chamber for one hour at 37°C. After washing for 5 minutes in two changes of 0.01 M PBS, pH 7.8, the cover slips are mounted in buffered glycerine and scanned with a 10X objective on a Zeiss microscope equipped with a dry darkfield condensor. Zeiss exciter filters BG 38 and BG 12/4 and barrier filters 41 and -65 are used.

Several uninoculated cultures are included in each group of tests. A positive

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*Pharmacia Fine Chemicals, Inc., Piscataway, New Market, N. J.
**Schleicher and Schuell & Co., Keene, N. H.
***Calbiochem, Los Angeles, California.
****Baltimore Biological Laboratory, Inc., Baltimore, Md.
result in the uninoculated controls is sometimes observed. Whenever this is found the entire batch of tests is repeated in a new preparation of BEK cells. Positive control cover slips prepared from several BVD strains are also stained.

RESULTS

A. SENSITIVITY AND SPECIFICITY OF THE CONJUGATE:

The conjugate prepared against New York 1 (NY 1) was first tested on a cytopathic strain of BVD virus (Oregon C24V) and 3 noncytopathic strains obtained from Dr. James Gillespie (Indiana 46, Iowa (Saunders), New York 1). All 4 strains exhibited cytoplasmic fluorescence in BEK cover slip cultures. Uninoculated cultures showed no fluorescence. Twelve cytopathic strains which had been isolated from specimens in stationary tube cultures in our laboratory also showed fluorescence. With 100 - 1000 TCID50 of these strains many large foci of fluorescent cells were observed 36-48 hours before cytopathic effects could be seen microscopically.

The specificity of the observed fluorescence was established by 'blocking' tests employing 2 different hyperimmune sera. One, of bovine origin, had been prepared against a noncytopathic BVD strain (NY 1), and the other, of rabbit origin, had been prepared against a cytopathic strain (Oregon C24V). Both of these sera substantially reduced or eliminated the fluorescence seen with all of the 16 above-mentioned strains. Normal bovine serum did not affect the intensity of the fluorescence. These results indicated that all of the strains, 12 cytopathic and 4 noncytopathic, were similar enough antigenically to be detected with the NY 1 conjugate. The conjugate did not give positive FA tests with IBR, PI-3, or several bovine enteroviruses.

The FA test was then applied to a group of tissues which had been preserved at -70°C for 6 - 18 months. These materials were from cases that had clinical histories compatible with BVD infection and had shown typical post mortem lesions. However, cytopathic BVD virus had not been isolated in stationary tube cultures from any of the specimens. Tissues from 29 cases were tested; 7 cases (24%) were positive by the FA method. Four of these were noncytopathic strains even after several blind passages; the other 3 proved to be cytopathic only after 2 or more blind passages in stationary cultures. Judging from our subsequent experience it is probable that the long period of storage would have reduced the number of virus identifications made by the FA method on this group of frozen specimens.

B. TYPES OF FLUORESCENCE:

Two types of cytoplasmic fluorescence were observed with different strains of BVD virus when using NY 1 conjugate. Some strains consistently produced a diffuse ‘cloudy’ fluorescence resembling that produced by a soluble antigen (Figure 1). With other strains the diffuse type of fluorescence was seen on the first or second day after inoculation into BEK cells, but by the third or fourth day, many small bright discrete particles had developed giving the cytoplasm a ‘speckled’ appearance (Figure 2). The ability to elicit either this particulate fluorescence or the diffuse type with NY 1 conjugate was a stable characteristic of individual virus strains and apparently was not correlated with their cytopathic
or noncytopathic capacity. Both types of fluorescence were specific as demonstrated by blocking tests with hyperimmune BVD antiserum. Slight variations in the intensity of fluorescence were seen with different virus strains but in no cases were the FA readings equivocal.

The diffuse type of fluorescence, but of diminished intensity, was also observed with 3 strains of BVD virus when they were stained with hog cholera conjugate (supplied by National Animal Disease Laboratory, Ames, Iowa). These same 3 strains exhibited a brilliant particulate type of fluorescence when stained with NY 1 conjugate. Cell line PK-15 (pork kidney)* when infected with hog cholera virus stained diffusely with the anti-BVD conjugate.

Although foci of fluorescent cells could be observed in cover slip cultures as early as 24 hours after inoculation with some specimens (Figure 3), with others, 2-3 days of incubation were required. Experience with a large number of FA positive specimens (fresh and stored for 1½ years at -70°C) indicated that a 4-day incubation period was optimal. After this incubation period either large foci or almost all cells in the monolayer were infected.

C. FA TESTS ON ROUTINE SPECIMENS, MARCH, 1968 TO MARCH, 1969:

1. Calves and older animals.

Bovine specimens on which virus isolation studies were requested or which were accompanied by a pertinent clinical history were tested by the FA method. Some clinical specimens (blood, nasal swabs, feces) were submitted by mail by practicing veterinarians; others were obtained on field trips by the laboratory diagnosticians. Post mortem tissues were obtained at autopsies performed at the farm or laboratory.

A total of 360 field cases involving calves and older animals were examined. Of these cases, 69 (19%) were found to have BVD virus infections by the FA method (Table 1). (In the year prior to this study, BVD virus was isolated and identified by cytopathic effects in tissue culture in 10-12% of the cases.)

During the first 2½ months of this study all specimens were tested concurrently by the FA method and by the tissue culture (BEK) method. A total of 26 cases of BVD virus infections were recognized by the FA test; in only 7 (28%) of these was an unequivocally cytopathic BVD strain isolated after a single passage in tissue culture.

860 specimens were tested and the numbers and types that were positive by FA tests are shown in Table II. The best source of BVD virus from sick animals was 'acute stage' blood. 15.4% of the blood samples tested were FA positive, followed by 9.3% of the nasal swabs and 4.6% of the fecal specimens. Where 100 or more post mortem cases were studied, the highest percentages of positive FA tests were obtained on spleen (18.8%) and intestine (17.5%).

The greatest number of BVD cases detected in 1968 by the FA test occurred from March through July with a second lesser peak occurring in November and December.

2. Aborted fetuses.

During the year of this study, fetal tissues from 90 field cases of bovine

*supplied by NADL, Ames, Iowa.
abortion were tested for the presence of BVD virus by the FA tissue culture method. Tissues from 14 (15.5%) of these cases were FA positive (Table I). One or more specimens of all the different fetal viscera examined was found to contain BVD virus (Table III). One out of 14 specimens of placental cotyledons was also FA positive. (After these results were compiled, BVD virus was identified in another placenta. In neither of these two cases could the virus be demonstrated in the viscera of the aborted fetus.)

During the 2½ month period when specimens were tested by the FA method and in tissue culture, BVD virus was identified by the FA method in 6 aborted fetuses. No cytopathic strains were found by the tissue culture method.

D. FA TESTS ON ORGANS USED FOR PRIMARY TISSUE CULTURES:

The FA tests identified BVD virus on secondary cover slip cultures in 11 (16.2%) of 68 pairs of bovine embryonic kidneys obtained from a local meat packing plant for use in preparing tissue cultures. This situation is detected as a positive FA test result on the uninoculated cultures which are always included with each batch of tests. FA tests on frozen sections of some of the original kidneys gave inconsistent results and this approach was therefore not used for identifying kidneys with BVD virus.

Six of 7 cell lines started from different pools of bovine embryonic testicles were also found to be 'carrying' BVD virus when FA tests were done on secondary or later passages of these cells. (The frequency of positive cell lines from pools of testes is not comparable to the frequency seen with the kidney cultures since the kidneys were from single animals.)

DISCUSSION

Nearly twice as many cases of BVD have been identified in our laboratory since the FA test described in this report has been used routinely as were identified in previous years by tissue culture isolation methods. Comparative tests establish that at least part of this increased incidence is attributable to the ability of the FA test to detect noncytopathic strains which previously were not recognized. Although the specific conjugate was prepared from an antiserum against a noncytopathic strain of BVD virus, it appears to be equally sensitive in identifying cytopathic strains. This suggests that there are antigenic similarities between different strains of the virus which are unrelated to their ability to produce cytopathic effects. This observation has also been made by other workers. In view of our later findings that bovine embryonic kidney cultures may 'carry' BVD virus, the possibility exists that the NY 1 antigen which we propagated in these cells and used for preparing specific conjugate was inadvertently 'contaminated' with other BVD virus strains. This might conceivably have increased the sensitivity of our conjugate by broadening its antigenic spectrum if indeed major antigenic differences between strains do occur. More relevant is the finding that strains of BVD virus probably share at least one antigenic component. This would make the FA test capable of recognizing all strains. Significantly, Gutekunst et al describe an apparently soluble complement-fixing antigen common to both a noncytopathic and a cytopathic strain. In our studies 'cloudy' diffuse cytoplasmic fluorescence was observed as the earliest immuno-
fluorescent event with all BVD virus strains and with some strains this was the only type of fluorescence seen. This early immunofluorescent antigen may be the same as the common soluble CF antigen previously described by Gutekunst et al.\(^7\) The 'speckled' particulate type of fluorescence, which we observed as a later development with some strains may represent antigens of the virus itself.

Other investigators have demonstrated an antigenic relationship between hog cholera and BVD viruses which appeared to be based on the presence of a common soluble antigen.\(^7,8\) Our work confirms this relationship and also suggests that a soluble antigen is involved since only the 'cloudy' type of fluorescence was seen when BVD virus strains were stained with hog cholera conjugate and vice versa.

BVD virus has long been considered as a possible cause of abortion in cattle\(^9\), although the only report known to us of the isolation of BVD virus from fetal tissues was made by Gillespie et al.\(^10\) They identified virus (noncytopathic) in 2 of 54 (3.7%) aborted fetuses by the 'cellular resistance' technique. With our FA test 14 of 90 (15.5%) fetuses from abortion cases were found to be infected with BVD virus. The higher percentage of infected fetuses in our study reflects either a greater incidence of infection and/or a greater sensitivity of the FA test. Six of 14 isolates detected in aborted fetuses were not cytopathic when studied in tissue culture. Further work is in progress to determine whether any of the 8 other isolates identified in fetal tissues by the FA test are cytopathic.

Our results apparently support field observations that BVD virus is a cause of bovine abortions, although to establish this, more work on experimental production of abortions is required.

Three possibilities can be considered to explain the finding that uninoculated control cultures from 11 of 68 pairs of bovine embryonic kidneys were infected with BVD virus. These are: one, that the relevant fetal kidneys obtained from a meat packing plant were already 'carrying' the virus; two, that the commercial fetal calf serum used for growing the cultures before they were used in tests included a serum from a viremic fetus; three, that the 'contamination' occurred in our laboratory. The last possibility seems most unlikely since we have never encountered tissue cultures 'contaminated' with other bovine viruses commonly used in our laboratory. The second possibility must be considered since the membrane filtration used to sterilize commercially prepared sera would not necessarily remove all virus particles. The most likely explanation in our opinion is that the fetuses from the meat packing plant from which the kidneys were obtained were already infected. Conceivably, these fetuses might have aborted at a later stage. Regardless of the source of the virus in these uninoculated control cultures, the results of FA tests on specimens are valid since a positive test on a specimen is accepted only when the uninoculated control cultures are negative.

The frequency with which bovine embryonic kidneys used for preparing tissue cultures were infected with noncytopathic BVD virus was an unexpected finding having widespread implications. The reliability of previously available procedures for detecting BVD virus, including both indirect methods for noncytopathic strains and tissue culture isolation methods for cytopathic strains, is predicated on the assumption that adventitious virus is not present in the tissue culture system employed. The FA method is demonstrably able to circumvent at
least part of this possible inherent error in the test system by its ability to recognize infected cells in the absence of cytopathic effects. For this reason, and because it is sensitive, specific and rapid, the FA test is recommended as the procedure of choice for the laboratory diagnosis of BVD virus infections.

SUMMARY

A fluorescent antibody tissue culture method for detecting BVD virus has been developed and applied to specimens from field cases. Over a period of a year, 69 (19%) of 360 cases involving calves or older animals were diagnosed as BVD virus infections by the FA test. Fetal tissues from 14 (15.5%) of 90 cases of bovine abortion were also found to contain BVD virus. The FA test is sensitive and specific in recognizing both cytopathic and noncytopathic strains. It also circumvents the complexities of possible adventitious virus and is therefore recommended for the laboratory diagnosis of BVD infection.
Fig. 1. A typical positive FA test showing the 'cloudy' diffuse type of cytoplasmic fluorescence. The bovine embryonic kidney culture was stained with NY 1 conjugate after inoculation with splenic tissue from a calf suspected of having BVD. Note the vacuoles in the cytoplasm produced by this particular isolate which also happened to be cytopathic. Approx. x 400
Fig. 2  A typical positive FA test showing the 'speckled' particulate type of cytopathic fluorescence. The bovine embryonic kidney culture was stained with NY 1 conjugate after inoculation with acute serum from a calf suspected of having BVD.  
Approx. x 400
Fig. 3  A lower power view of the culture illustrated in Fig. 2. Note the foci of cells showing the 'speckled' type of fluorescence. At this early stage of infection some individual cells still show only 'cloudy' fluorescence.

Approx. x 100
### TABLE I

RESULTS OF FA TESTS FOR BVD VIRUS ON FIELD CASES, MARCH 1968 TO MARCH 1969

<table>
<thead>
<tr>
<th></th>
<th>Total Cases Tested</th>
<th>FA Tested Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves and Older Animals</td>
<td>360</td>
<td>69</td>
<td>19.0</td>
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<tr>
<td>Aborted Fetuses</td>
<td>90</td>
<td>14</td>
<td>15.5</td>
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### TABLE II

RESULTS OF FA TESTS FOR BVD VIRUS IN SPECIMENS FROM CALVES AND OLDER ANIMALS

<table>
<thead>
<tr>
<th>Specimens Tested</th>
<th>Total Number Tested</th>
<th>FA Tested Positive</th>
<th>% Positive</th>
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<tr>
<td>Acute serum</td>
<td>130</td>
<td>20</td>
<td>15.4</td>
</tr>
<tr>
<td>Nasal swabs</td>
<td>182</td>
<td>17</td>
<td>9.3</td>
</tr>
<tr>
<td>Feces</td>
<td>215</td>
<td>10</td>
<td>4.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>138</td>
<td>26</td>
<td>18.8</td>
</tr>
<tr>
<td>Intestine</td>
<td>114</td>
<td>20</td>
<td>17.5</td>
</tr>
<tr>
<td>Abomasum</td>
<td>24</td>
<td>4</td>
<td>16.6</td>
</tr>
<tr>
<td>Lung</td>
<td>22</td>
<td>5</td>
<td>22.7</td>
</tr>
<tr>
<td>Liver</td>
<td>19</td>
<td>2</td>
<td>10.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>12</td>
<td>4</td>
<td>33.3</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2</td>
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</tr>
<tr>
<td>Thoracic fluid</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Total = 860
FA Tested Positive = 108
% Positive = 12.5

### TABLE III

RESULTS OF FA TESTS FOR BVD VIRUS IN TISSUES FROM ABORTED FETUSES

<table>
<thead>
<tr>
<th>Fetal Tissues Tested</th>
<th>Total Number Tested</th>
<th>FA Tested Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>89</td>
<td>8</td>
<td>9.0</td>
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<tr>
<td>Kidney</td>
<td>27</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>Liver</td>
<td>23</td>
<td>1</td>
<td>4.3</td>
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<tr>
<td>Lung</td>
<td>22</td>
<td>5</td>
<td>22.7</td>
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<tr>
<td>Pleural fluid</td>
<td>13</td>
<td>2</td>
<td>15.4</td>
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<tr>
<td>Testes</td>
<td>7</td>
<td>1</td>
<td>14.3</td>
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<tr>
<td>Placental cotyledons</td>
<td>14</td>
<td>1</td>
<td>7.1</td>
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Total = 195
FA Tested Positive = 20
% Positive = 10.3
REFERENCES


IN VITRO STUDY OF BOVINE VIRUS DIARRHEA VIRUS:
INTERACTIONS WITH SOME SELECTED VIRUSES*

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University of Guelph

INTRODUCTION:
It is now generally recognized that two different viruses can infect a given susceptible cell culture, and may result in a dual infection of the cell culture, or one virus may either interfere with, or enhance, the replication of the other virus. Some of these phenomena have been observed and reported with Bovine Virus Diarrhea (BVD) virus. For example, cell cultures infected with non-cytopathic virus strains of BVD are resistant to challenge with a cytopathic strain\(^1,2\); on the other hand observations by Inaba et al\(^3\) led to the recognition of the Exaltation of Newcastle Disease Virus (END Effect) by BVD virus. The present report describes experiments to determine the ability of BVD virus to manifest these phenomena in its interaction with selected viral agents that cause diseases which may be easily confused with BVD clinically.

MATERIALS AND METHODS:

Cell Culture – Embryonic bovine spleen (EBS) cell cultures were prepared by trypsinizing a fetal spleen; the cell suspension was seeded into 32 oz Blake bottles and was grown and maintained in Minimum Essential Medium with Earles balanced salt solution, containing 20% and 5% fetal calf serum respectively. The monolayer appeared at first to be composed of fibroblast-like cells, these were subsequently replaced in about 3-4 weeks by epithelial-like cells and the monolayers were then ready for subculture.

Virus stocks – The viruses used in these experiments were the cytopathic Oregon C24V strain** (9th passage) and the noncytopathic New York strain** (9th passage) of BVD virus. Infectious Bovine Rhinotracheitis (IBR)** virus (5th passage) and Parainfluenza 3*** (SF-4, 9th passage) virus isolates.

Stocks of each were prepared by propagation in EBS cell cultures.

BVD-IBR Virus Infected Cell Cultures – The difference in growth rates of the IBR and BVD viruses made it necessary to design these experiments so that the monolayer was not completely destroyed at those times selected for examination. The following procedures were adopted.

EBS cells grown on coverslips were inoculated with 0.1 ml. of \(10^6\) /ml of cytopathic Oregon virus strain. Another set of cell cultures was similarly

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*The senior author is supported by a Colombo Plan Scholarship. The investigation was partially supported by a NRC Grant (A-1389).
**Supplied through the kindness of Dr. A. Fernelius, National Animal Disease Laboratory, Ames, Iowa.
***Isolated and identified by one of the authors (M.S.) from cattle in Ontario.
inoculated with 0.1 ml of $10^6$/ml of noncytopathic New York strain. On day 1 and 3 post inoculation 20 tubes from each set were challenged with 0.1 ml of 100 CCID$_{50}$ ($10^4$/ml) of IBR virus. Uninoculated cultures, as well as cultures infected with either IBR or BVD virus were included as controls. On day 7 the cell monolayers were fixed and stained with hematoxylin and eosin and acridine orange. The culture fluids were pooled and aliquots were titrated for BVD and IBR viruses.

Duplicate sets of half log dilutions from 0.9 to 7.0 were made of the infected fluids in phosphate buffered saline. Equal volumes of a known anti-BVD serum were added to one set and an anti-IBR serum to the other. The virus antibody mixtures were incubated for 1 hour at 37°C and a titration was carried out in EBS cell cultures by inoculating each of 4 tubes with 0.2 ml of every dilution. Titration of the New York virus strain was carried out by the Interference test. Briefly this is done by inoculating EBS monolayers with 0.2 ml of the virus antibody dilution. Five days post inoculation the cultures were challenged with 0.1 ml of 100 CCID$_{50}$ of cytopathic Oregon virus strain. Cells infected by the noncytopathic virus were resistant to the challenge of the cytopathic virus strain.

BVD-SF$_4$ Virus Infected Cultures – Similar experiments were carried out using 0.1 ml of 1 CCID$_{50}$ ($10^4$/ml) of SF$_4$ virus in the place of IBR for challenge either simultaneously, or on day 1 or day 3 of BVD infected cultures. In another experiment Leighton tubes were first inoculated with 0.1 ml of 1 CCID$_{50}$ of SF$_4$ virus and later challenged with 0.1 ml of $10^6$ concentration of New York strain of BVD virus on day 1 or 3. Cell cultures inoculated with SF$_4$ or BVD viruses were included as controls. On day 7 the cultures were fixed and stained with hematoxylin eosin. The culture fluids were pooled and titrated for BVD virus or SF$_4$ virus hemagglutinin. Hemagglutination test (HA) using 0.3% bovine red blood cells was selected for titration of SF$_4$ virus hemagglutinin. The BVD virus yield was titrated in EBS cell cultures after neutralizing the SF$_4$ virus with specific antiserum.

**RESULTS**

**BVD-IBR virus infected cell cultures**

Cytological changes – on the basis of morphological evidence the cell cultures infected with Oregon virus strain were fully susceptible to challenge with IBR virus. Observation indicates that some cells were infected with either BVD or IBR virus while other cells were doubly infected. The CPE attributed to the infecting viruses were readily differentiated from each other. The vacuoles and eosinophilic inclusions in the cytoplasm were due to BVD virus while the nuclear changes and the rounding and stranding effects on the cells were IBR induced. The nucleus was shrunken, pyknotic and sometimes contained eosinophilic inclusions. In the double infected cells both of these changes were observed (Fig. 1). With acridine orange staining the nuclei of the double infected cells contained numerous granules of bright green fluorescence. The cytoplasm showed an intense reddish orange color particularly around the nucleus. In some cells in the advanced stage of infection the numerous nuclear granules fused into a large mass of greenish yellow fluorescence while in the cytoplasm some cytoplasmic vacuoles were observed.
The cells infected with the New York virus strain seemed to be resistant to the challenge infection of IBR virus. There was no CPE observed in hematoxylin eosin stained monolayers regardless of the time of IBR challenge.

With acridine orange staining the nucleus did not show any change and took the staining characteristics of uninfected cells. The cytoplasm however showed an increased intensity of reddish orange fluorescence compared to the cells of the uninoculated control culture.

**Virus Yield** – The yields of BVD and IBR virus are presented in Table 1. The titer of the Oregon virus strain obtained from the doubly infected cell cultures was not significantly different from the virus control. This suggests that the replication of the Oregon virus strain was not impaired in the doubly infected cells. The IBR virus titer also suggests that it was capable of replicating without interference, in the cells already infected with the Oregon virus strain. The titer of the New York virus strain in the cultures challenged with IBR virus was as high as that of the virus control as determined by the interference test while there was not detectable IBR virus. This finding suggests that the IBR virus failed to infect and replicate in the cell cultures already infected with non-cytopathic New York virus strain.

**BVD-SF₄ Virus Infected Cell Cultures**

**Cytological Changes** – In the cell cultures infected with SF₄ and challenged simultaneously or on day 1 with Oregon or New York virus strains of BVD marked cytopathic changes (2-3+) were observed as early as four days. There were a few cells showing cytoplasmic eosinophilic inclusions. A few syncytial cells containing 3-8 nuclei were also present. There were no cytoplasmic vacuoles observed. Maximum CPE in this infected culture was observed on day 7. (Table 2). In the hematoxylin eosin stained monolayers, total destruction of the cells were observed. The cells disintegrated and the cytoplasm appeared as scattered small eosinophilic round masses and there was no discernible nuclear structure (Fig. 2). The BVD infected cell monolayers that were challenged with SF₄ virus on day 4 have the same type of CPE (2-3+) as described above. Similarly the appearance of the CPE was the same in the cell monolayers infected with SF₄ virus prior to BVD virus. The changes in the doubly infected monolayers observed on day 7 were not characteristic or typical of either BVD or SF₄ virus CPE. The SF₄ V infected controls however, showed only a few inclusions and syncytial formation on day 7.

**Titer of SF₄ Virus Hemagglutinin and BVD Virus** – Results of Hemagglutination titers of the infected culture fluid using 0.3% bovine red blood cells are presented in Table 2. On day 7 an HA titer of 16 was obtained from the Oregon or New York virus infected cultures which were challenged simultaneously or on day 1 with SF₄ virus. Those that were challenged with SF₄ on day 3 post-inoculation showed a titer of 8. On the other hand the HA titer obtained from cultures initially infected with SF₄ virus and challenged with the New York strain on day 1 and 3 were 16 and 4 respectively. The control culture infected with SF₄ had an HA titer of 2.

The result of BVD virus titration compared to the yield of the BVD virus control indicated that the BVD virus replication was not affected in the presence of SF₄ infection.
DISCUSSION

Cases of virus interaction with other viruses have been reported in literature. Virus replication in cell culture may be interfered with or intensified by coinfection with another virus. In some cases dual infection of the cell may result. The findings with the attempts to superinfect IBR virus in BVD virus infected cells showed that it is possible to infect the cell with both viruses, each virus producing its characteristic CPE (Fig. 1). The yield of Oregon and IBR viruses from the doubly infected culture also indicates that both viruses replicated. The virus titers however were not significantly different from their respective virus controls. There seemed to be no interference in the replication of either of the two viruses in double infected cultures. It should be pointed out however, that no attempts were made to determine the virus yields of a singly and doubly infected isolated cell.

In another circumstance, cells infected with New York virus strain were resistant to IBR virus challenge. In an attempt to explain this phenomenon the demonstration of interferon was undertaken. Pooled culture fluids from the resistant cultures were neutralized with anti-BVD serum and this neutralized fluid was then used to protect uninfected cell cultures from IBR virus infection. The failure of the culture fluid to confer protection or resistance to IBR virus suggests that interferon may not be involved. However, this unsuccessful attempt to demonstrate interferon in the culture fluids does not preclude the possible role of interferon in these resistant cultures.

The absence of detectable level of interferon may be due to the rapid turnover rate of interferon which reduces levels below detectability\(^6,7\). Stancek\(^8\) suggested the possibility that intracellular interferon in amounts which cannot be detected may still be highly effective. Rodriguez and Henle\(^9\) suggested that infection by a complete or incomplete virus may induce production of the proposed antiviral component of the interferon system without inducing interferon as such. It is possible that this may be the case in the resistant cultures infected by the noncytopathic New York virus strain. Furthermore, the noncytopathic virus strains may be capable in inducing interferon while the cytopathic strains are poor inducers in infected cells. This difference in the ability to induce interferon may explain the difference between the virulence of the cytopathic and noncytopathic virus strains of BVD in cell cultures. It has also been shown that cells infected with noncytopathic virus strains are refractory to challenge of cytopathic strains of BVD virus and this resistance has been explained as competition for receptor or replication sites\(^1,2\). However, the resistance of cells infected with cytopathic virus strains against the noncytopathic virus strains has not been demonstrated.

The results from cell cultures doubly infected with BVD and SF\(_4\) viruses showed that the enhancement of CPE and the increased HA titer were attained only after BVD virus had infected the cells for at least 3 days. It is believed that enhancement of SF\(_4\) virus infection requires BVD virus replication which in turn results in the inhibition of interferon production by SF\(_4\) virus. This mechanism has been suggested for the exaltation of Newcastle disease virus by hog cholera or BVD virus\(^10\). When BVD virus was neutralized with specific antisera before it was used as inoculum this enhancement phenomenon was inhibited, indicating
that this phenomenon is dependent on BVD virus infectivity. Isaacs et al\textsuperscript{11} suggested that the inhibition of interferon production may be mediated by an inhibitory factor produced by the cells upon coinfection with the helper virus or stimulating virus. These workers were able to demonstrate the presence of a substance which suppresses or inhibits interferon production. However, these findings are complicated by the discovery of an enhancer substance which is produced in cells upon viral infection and is capable of enhancing the virus replication\textsuperscript{12}. Brailovsky and Chany\textsuperscript{13} also suggested the role of another substance called stimulon for the enhancement of the replication of the coinfecting virus. At present we have very limited information about the nature and functions of any of these factors. They may be significant in the pathogenesis of BVD infection. For example, these factors may play an important role in the ability of BVD virus to enhance the action of other viruses.

The enhancement of benign or latent viruses may be responsible for the post vaccinal mucosal disease conditions that have been described\textsuperscript{14}.

The diagnosis of BVD virus in intercurrent or mixed infections by conventional methods is difficult because of the possible enhancement of the coinfecting virus. However, using direct fluorescent antibody staining methods the presence of BVD viral antigen could be detected even in the presence of an enhanced CPE\textsuperscript{15}.

Further studies of the described phenomena should serve to clarify these or similar observations.
TABLE 1: VIRUS YIELD AND CYTOPATHIC CHANGES IN DOUBLY INFECTED EMBRYONIC BOVINE SPLEEN CULTURES.

<table>
<thead>
<tr>
<th>BVD Virus</th>
<th>Time of IBR Challenge</th>
<th>Cytopathic changes at Day 7</th>
<th>BVD virus yield ( \log CCID_{50}/0.1 \text{ ml.} )</th>
<th>IBR virus yield ( \log CCID_{50}/0.1 \text{ ml.} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR</td>
<td>day 1</td>
<td>cells rounded and contracted, cytoplasmic strands extending from one cell to another. Nucleus shrunken and pyknotic, nuclear inclusions present (3-4+)</td>
<td>0.1 ml.</td>
<td>0.1 ml.</td>
</tr>
<tr>
<td>Oregon strain</td>
<td>---</td>
<td>cytoplasmic vacuolations of varying shapes and sizes (3-4+)</td>
<td>5.4</td>
<td>-</td>
</tr>
<tr>
<td>Oregon strain</td>
<td>day 1 post inoculation</td>
<td>Both IBR and BVD CPE discernible.</td>
<td>5.5</td>
<td>4</td>
</tr>
<tr>
<td>Oregon strain</td>
<td>day 3 post inoculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York strain</td>
<td>---</td>
<td>No CPE</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>New York strain</td>
<td>day 1 post inoculation</td>
<td>No CPE</td>
<td>3.87</td>
<td>-</td>
</tr>
<tr>
<td>New York strain</td>
<td>day 3 post inoculation</td>
<td>No CPE</td>
<td>4.06</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE 2: STIMULATING EFFECT OF BVD VIRUS TO THE HA TITER AND CPE OF SF4 VIRUS IN EMBRYONIC BOVINE SPLEEN CELL CULTURES.

<table>
<thead>
<tr>
<th>Initial Virus Infection</th>
<th>Time of Virus Challenge</th>
<th>Observation on Day 7 after Initial Infection</th>
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<tr>
<td></td>
<td></td>
<td>CPE</td>
</tr>
<tr>
<td>Oklahoma virus strain</td>
<td>Simultaneous with SF4 V</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Day 1 with SF4 V</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Day 3 with SF4 V</td>
<td>+++</td>
</tr>
<tr>
<td>New York virus strain</td>
<td>Simultaneous with SF4 V</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Day 1 with SF4 V</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Day 3 with SF4 V</td>
<td>++</td>
</tr>
<tr>
<td>SF4 Virus</td>
<td>Day 1 with NY virus strain</td>
<td>++++</td>
</tr>
<tr>
<td>SF4 Virus</td>
<td>Day 3 with NY virus strain</td>
<td>++</td>
</tr>
</tbody>
</table>

++++ 75-100% of cells affected  
+++ 50-75% of cells affected  
++ 25-30% of cells affected  
+ < 25% of cells affected
Fig. 1 EBS cell monolayer doubly infected with IBR and BVD virus. Arrows showing cells with both IBR and BVD virus CPE 420X.
Fig. 2 EBS cell monolayer doubly infected with BVD and SF₄ virus showing enhancement of SF₄ virus CPE 100X.
REFERENCES


INTRODUCTION
Animal disease reporting has been discussed for many years. The advent of the computer has provided us with a tool that makes animal disease reporting a reality. Funds are extremely important when providing for animal disease reporting systems, however, there is one other element that must be available—cooperation. The desire of the laboratory directors as well as the diagnosticians within the laboratory for information is the most important element of any disease reporting system.

Animal disease reporting is vitally needed in conjunction with the continuance of present research programs and the formulation of new research programs. Animal disease reporting will assist with safeguarding of our nation’s food supply, will provide information on economic costs due to animal loss, will provide information on seasonal and geographic disease trends as well as information on diseases transmissible from animals to man, will provide perspective for projection of future disease control and eradication programs and will help in the development of pharmaceutical and biological products.

DIAGNOSTIC LABORATORY DISEASE REPORTING
The American Association of Veterinary Laboratory Diagnosticians by its action at the annual meeting of the association in New Orleans in October 1968, has initiated a national computerized diagnostic laboratory disease reporting system. The system is designed to provide for the recording and subsequent retrieval of both disease and administrative information.

Diagnostic laboratory disease reporting is now under way and current laboratories using the system are located in Iowa, South Dakota, Kentucky, Wisconsin, Kansas, San Diego, and Indiana. Discussions are under way with several other laboratories to establish additional cooperators in the AAVLD Disease Reporting System.
AAVLD ANIMAL DISEASE REPORTING ABSTRACT

One of the prerequisites for computerized reporting of disease and administrative information is the development of a sheet which will provide for systematic recording of the data. Figure I shows the AAVLD abstract sheet. The information from the laboratory's case card is abstracted, coded and written on this sheet. A detailed explanation of how to complete this sheet is given below.

**Institution** — Each institution or diagnostic laboratory is assigned a specific code. This provides a unique identification of each laboratory and also provides a means of separating the data of several laboratories at a central processing agency. The first two digits of the code represent the state in which the laboratory is located and the second two digits are the actual number assigned to the laboratory.

**Accession Number** — This number is the accession number or case number assigned to the case by the laboratory. In most cases the laboratory starts with the number 00001 at the beginning of each year and consecutively numbers the cases as they are submitted to the laboratory.

**Report Date** — This section is used to record the month and the last digit of the year that the report is completed. Often a preliminary report may be sent to the client and even subsequent reports, however, most laboratories do not complete this abstract until the entire case is completed.

**County or Location** — This space is used to record the county or location from which the case was submitted. In the case of a county diagnostic laboratory, a code could be assigned to each geographical area within the county. The reason for this being recorded is to assist with showing the geographical location of disease problems.

**Species or Type of Specimen** — A code is entered to reflect the species of animal submitted to the laboratory. If a water sample or a feed sample is submitted to the laboratory, a code for these "types of specimen" can be entered in this space. If for example a bovine and a sample of water were both submitted, these can be recorded by putting the bovine on one sheet and recording the diagnoses and laboratory findings associated with the bovine and then on a second sheet record the same accession number, enter the code for the water sample, record the laboratory findings associated with the water sample and check the item at the bottom of the abstract saying "x Yes this is a continuation sheet". This method provides for recording all the information, however, does not inflate your case load figures by calling these two cases instead of one case with both a bovine and a water sample submitted.

**Number of Specimen(s)** — This section is used for recording administrative information. The number of animals submitted is recorded. If a whole animal and a liver and spleen are submitted, this is considered two animals. If three livers, two kidneys, a spleen and several pieces of intestine are submitted, this is considered to be three animals. The number of non-animal samples is recorded also. A bale of hay, 50 pounds of feed, and a gallon of water would be recorded as three non-animal samples.

**Sex** — The applicable category is checked.

**Age** — The applicable category is checked.

**Animal Breed** — The breed of animal is entered and the code is recorded.

**Morbidity and Mortality Data** — This space is used to record the number of
animals in the affected pen or group and the number sick and the number dead in relation to this particular case. A maximum of 9,999 animals can be recorded on one sheet and if there are more than 10,000 animals in a particular pen affected, then the balance of the animals can be put on a subsequent sheet using the same accession number and checking that the second sheet is a continuation sheet as mentioned above. The same is true for recording the number sick and the number dead.

**Diagnoses or Laboratory Findings** — This space is used to record the primary and secondary diagnoses or laboratory findings associated with the case. If the diagnostician is able to make a diagnosis regarding the case then the proper diagnostic code is entered. If, however, he has only laboratory results or findings then these codes are entered. An example of this situation might be that a whole animal was submitted to the laboratory along with a good history regarding the case and the diagnostician diagnosed the case as “Pasteurellosis”. If, however, he had received no history and only a piece of lung tissue from which he isolated *Pasteurella* sp., he might record only his laboratory findings as “Bacterial Examination — *Pasteurella* sp.”

**Administrative Information** — This section is used to record the number of virus isolation attempts, the number of chemistry and toxicology tests conducted, the number of histopathology examinations, and the number of bacteriological examinations conducted in conjunction with handling of the case.

**Serology Results** — The serological tests conducted in the laboratory as well as the results of these tests are recorded in this space. Codes have been established for some of the serological tests and other codes are added as needed. Other types of laboratory tests can also be recorded as long as the results of the tests can be recorded as positive or negative. The only requirement is to establish a code.

**Additional Diagnoses or Laboratory Findings** — This section is used to record additional diagnoses or laboratory findings. It is handled in reporting exactly as the above diagnoses section.

**Final Report** — This is used to assist the laboratory director in determining the length of time required to process the cases submitted to the laboratory. This section is used in any way desired, however, the number of days recorded is usually the length of time required to completely process the case.

**Is This a Continuation Sheet** — The appropriate item is checked and this serves the function of providing a mechanism for the recording of all information concerning a case. In counting cases for the monthly report, those sheets marked “Continuation — yes” are not figured into the total cases for the month.

The AAVLD abstract has been developed in consultation with several diagnostic laboratory directors. There is much additional information that could be gathered — both of a disease nature and of an administrative nature. If a particular laboratory desires to gather more data, this can be done by adding supplemental sheets to provide for the systematic recording of additional data. The computer in turn can pull all the sheets together through the use of the common accession number.

**CODES USED IN THE AAVLD SYSTEM**

Codes have been developed for each participating laboratory, the counties
within each state, the species of animal, the breeds of animals, the diagnoses and laboratory findings, and the serological tests. Appendix A shows a sample of the codes in use.

If there is a diagnosis or laboratory finding that is not on the list, the next consecutive code is assigned. This provides an open-ended mechanism and allows for the inclusion of new terms as the need arises. The same is true for the “species of animal” or “type of specimen” codes.

REPORTS FOR THE AAVLD SYSTEM

Figure 2 is a sample report of the number of diagnoses made or laboratory findings recorded in each species of animal submitted to the laboratory for the month. This data is also summarized yearly for the laboratory.

It is not economically feasible to record all data that is pertinent to a case such as the history, clinical signs, vaccination procedures used and many other items of information. For this reason the primary information reported is the diagnoses by species of animal. However, it is highly desirable to have a mechanism to peruse the actual case report if necessary. For this reason the report shown in Figure 3 is produced at the request of the laboratory director. This report shows the diagnoses and laboratory findings by species of animal and also shows the accession numbers associated with the diagnoses. For in-depth research on a particular disease, the actual case report can be pulled easily by using this report.

The administrative data reported is shown in Figures 4, 5, and 6. These reports show the number of animals submitted to the laboratory by species of animal, the number and results of the serological tests conducted by species of animal, the number of accessions by species of animal, the number of accessions by county, the lag time for reporting (that is the number of cases and how many days were required to complete each case), a comprehensive rabies report and an administrative summary for the reporting period.

SUMMARY

Animal disease reporting from veterinary diagnostic laboratories is now a reality. The AAVLD can be proud that it is started and has done so on very limited funds. The system works and has proven successful in several laboratories. It should be maintained and constantly improved. It will provide information to those interested in animal diseases and will provide the laboratory directors with disease information, with administrative information and with the capability to get at the fine details of the case through retrieval of the accession number.
American Association of Veterinary Laboratory Diagnosticians
Animal Disease Reporting Abstract

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<th>County or Location</th>
<th>Species or Type of Specimen</th>
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<td>1 2 3 4</td>
<td>5 6 7 8 9</td>
<td>10 11 12</td>
<td>13 14 15</td>
<td>16 17 18</td>
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</tbody>
</table>

<table>
<thead>
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<th>Age</th>
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</thead>
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<td>22 23 24</td>
<td></td>
<td></td>
</tr>
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</table>

<table>
<thead>
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<th>Animal Breed</th>
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<tr>
<td>27 28 29</td>
<td>No. of Animals in Affected Group</td>
</tr>
<tr>
<td></td>
<td>30 31 32 33</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Diagnoses or Laboratory Findings</th>
<th>Administrative Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Diagnosis</td>
<td>No. of Virus Isolation Attempts</td>
</tr>
<tr>
<td></td>
<td>No. of Chemistry &amp; Toxicology Tests</td>
</tr>
<tr>
<td>Second Diagnosis</td>
<td>No. of Histopath Exams</td>
</tr>
<tr>
<td></td>
<td>No. of Bact. Exams</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serology Results</th>
<th>Additional Diagnoses or Laboratory Findings</th>
<th>Final Report (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Code</td>
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<tr>
<td>No. Positive</td>
<td>54 55</td>
<td>68 69 70 71</td>
</tr>
<tr>
<td>No. Negative</td>
<td>56 57 58</td>
<td>72 73 74 75</td>
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<tr>
<td>No. Suspect</td>
<td>59 60 61</td>
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<tr>
<td>No. Unsuitable</td>
<td>62 63 64</td>
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<td></td>
<td>65 66 67</td>
<td></td>
</tr>
</tbody>
</table>

Is this a continuation sheet? __ No (0) __ Yes (1)

Figure 1.
## DISEASE REPORTING SYSTEM

### AAVLD NATIONAL ANIMAL DISEASE REPORTING SYSTEM

**July 1969**

### Diagnoses or Findings

<table>
<thead>
<tr>
<th>Species</th>
<th>Diagnoses or Findings</th>
<th>Number of Diagnoses or Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CANINE</strong></td>
<td>Rabies Positive-Human Exposure</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rabies Positive-No Human Exposure</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rabies Negative-Human Exposure</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rabies Negative-No Human Exposure</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Distemper</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Abscess</td>
<td>3</td>
</tr>
<tr>
<td><strong>PORCINE</strong></td>
<td>Abortion-Leptospirosis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bact-<em>Salmonella</em> species</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Anemia-Baby Pig</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Vibrionic Dysentery</td>
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<tr>
<td></td>
<td>Hog Cholera</td>
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</tr>
<tr>
<td></td>
<td>Erysipelas</td>
<td>7</td>
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<tr>
<td><strong>BOVINE</strong></td>
<td>Rabies Positive-Human Exposure</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rabies Positive-No Human Exposure</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rabies Negative-Human Exposure</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rabies Negative-No Human Exposure</td>
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<tr>
<td></td>
<td>Infectious Bovine Rhinotracheitis</td>
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<td>Bovine Virus Diarrhea</td>
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<td>Polioencephalomalacia</td>
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<tr>
<td></td>
<td>Abortion-Leptospirosis</td>
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<td><strong>EQUINE</strong></td>
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<td>Rabies Positive-No Human Exposure</td>
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<td></td>
<td>Rabies Negative-No Human Exposure</td>
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<tr>
<td></td>
<td>Infectious Equine Anemia</td>
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<td></td>
<td>Strangles</td>
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<td><strong>CHICKEN</strong></td>
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<td>Coccidiosis</td>
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<td>Fowl Pox</td>
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<tr>
<td></td>
<td>Fowl Cholera</td>
<td>5</td>
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</table>

Figure 2
### AAVID NATIONAL ANIMAL DISEASE REPORTING SYSTEM

<table>
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<th>Species</th>
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<td>Pneumonia</td>
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<tr>
<td>Distemper</td>
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<tr>
<td>Distemper</td>
<td>BOVINE</td>
<td>3</td>
</tr>
<tr>
<td>Bovine Virus Diarrhea</td>
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<td>2</td>
</tr>
<tr>
<td>Mastitis</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Polioencephalomalacia</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hog Cholera</td>
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<td>6</td>
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<td>Erysipelas</td>
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<td>5</td>
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<tr>
<td>Vibrionic Dysentery</td>
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<td>3</td>
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<tr>
<td>Abscess/Cervical</td>
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<td>2</td>
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</tbody>
</table>

**Figure 3**
### Species of Animal

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Animals Submitted</th>
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<tbody>
<tr>
<td>Canine</td>
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<tr>
<td>Feline</td>
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</tr>
<tr>
<td>Equine</td>
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</tr>
<tr>
<td>Porcine</td>
<td>47</td>
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<tr>
<td>Bovine</td>
<td>122</td>
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<tr>
<td>Chicken</td>
<td>75</td>
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<tr>
<td><strong>Total Animals Submitted</strong></td>
<td><strong>268</strong></td>
</tr>
</tbody>
</table>

### Serological Tests

#### Species = Bovine

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
<th>Suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis</td>
<td>10</td>
<td>575</td>
<td>0</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>13</td>
<td>4075</td>
<td>0</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td>4</td>
<td>105</td>
<td>0</td>
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<tr>
<td><strong>Total</strong></td>
<td>27</td>
<td>4755</td>
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### Species of Animal

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Accessions</th>
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</thead>
<tbody>
<tr>
<td>Canine</td>
<td>13</td>
</tr>
<tr>
<td>Feline</td>
<td>7</td>
</tr>
<tr>
<td>Equine</td>
<td>4</td>
</tr>
<tr>
<td>Porcine</td>
<td>10</td>
</tr>
<tr>
<td>Bovine</td>
<td>97</td>
</tr>
<tr>
<td>Chicken</td>
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<tr>
<td><strong>Total Accessions</strong></td>
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</tbody>
</table>

Figure 4
AAVLD NATIONAL ANIMAL DISEASE REPORTING SYSTEM

<table>
<thead>
<tr>
<th>County</th>
<th>Number of Accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adair</td>
<td>10</td>
</tr>
<tr>
<td>Adams</td>
<td>10</td>
</tr>
<tr>
<td>Benton</td>
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</tr>
<tr>
<td>Linn</td>
<td>6</td>
</tr>
<tr>
<td>Jones</td>
<td>12</td>
</tr>
<tr>
<td>Cedar</td>
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</tr>
<tr>
<td><strong>Total Accessions</strong></td>
<td><strong>63</strong></td>
</tr>
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</table>

Lag Time for Reporting

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<th>Number of Accessions</th>
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<tr>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
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<tr>
<td>3</td>
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<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

COMPREHENSIVE RABIES SUMMARY

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<thead>
<tr>
<th>Species of Animal</th>
<th>Rabies Positive</th>
<th>Rabies No Human</th>
<th>Rabies Negative</th>
<th>Rabies No Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human Exposure</td>
<td>No Human Exposure</td>
<td>Human Exposure</td>
<td>No Human Exposure</td>
</tr>
<tr>
<td>Canine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Feline</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Porcine</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Equine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bovine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td><strong>5</strong></td>
<td><strong>4</strong></td>
<td><strong>5</strong></td>
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ADMINISTRATIVE SUMMARY

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<tbody>
<tr>
<td>Number of Non-animal Samples</td>
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</tr>
<tr>
<td>Number of Virus Isolation Attempts</td>
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</tr>
<tr>
<td>Number of Chemistry and Toxicology Tests</td>
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</tr>
<tr>
<td>Number of Histopathology Exams</td>
<td>23</td>
</tr>
<tr>
<td>Number of Bacteriology Exams</td>
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<tr>
<td>Number of Accessions</td>
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Figure 5

Figure 6
# APPENDIX A

## CODES USED IN THE AAVLD DISEASE REPORTING SYSTEM

**Diagnostic Laboratories**

<table>
<thead>
<tr>
<th>Code</th>
<th>Laboratory</th>
</tr>
</thead>
</table>
| 1401 | Veterinary Diagnostic Laboratory  
Iowa State University, Ames, Iowa |
| 4001 | Veterinary Diagnostic Laboratory  
South Dakota State University, Brookings, South Dakota |
| 1603 | Veterinary Diagnostic Laboratory  
State of Kentucky, Hopkinsville, Kentucky |
| 1501 | Veterinary Diagnostic Laboratory  
Kansas State University, Manhattan, Kansas |
| 4801 | Veterinary Diagnostic Laboratory  
State of Wisconsin, Madison, Wisconsin |
| 0408 | San Diego County Diagnostic Laboratory  
San Diego, California |
| 1301 | Veterinary Diagnostic Laboratory  
Purdue University, Lafayette, Indiana |
<table>
<thead>
<tr>
<th>Code</th>
<th>Diagnoses or Laboratory Finding</th>
<th>Code</th>
<th>Diagnoses or Laboratory Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Abortion-IBR</td>
<td>555</td>
<td>Neo-Trichoepitheloma</td>
</tr>
<tr>
<td>18</td>
<td>Abortion-Nitrate</td>
<td>579</td>
<td>Panleucopenia</td>
</tr>
<tr>
<td>28</td>
<td>Aleutian Disease</td>
<td>582</td>
<td>Para-Balantidium coli</td>
</tr>
<tr>
<td>38</td>
<td>Anaplasmosis</td>
<td>591</td>
<td>Para-Demodex bovis</td>
</tr>
<tr>
<td>40</td>
<td>Anemia-Infectious Feline</td>
<td>597</td>
<td>Para-Echinococcus species</td>
</tr>
<tr>
<td>83</td>
<td>Bact-Bacillus species</td>
<td>600</td>
<td>Para-Fasciola species</td>
</tr>
<tr>
<td>124</td>
<td>Bact-Pasteurella hemolytica</td>
<td>668</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>125</td>
<td>Bact-Pasteurella multocida</td>
<td>670</td>
<td>Pneumonia-Bacterial</td>
</tr>
<tr>
<td>135</td>
<td>Bact-Salmonella cerro</td>
<td>676</td>
<td>Pneumonia-Inhalation</td>
</tr>
<tr>
<td>136</td>
<td>Bact-Salmonella chester</td>
<td>677</td>
<td>Pneumonia-Parasitic</td>
</tr>
<tr>
<td>139</td>
<td>Bact-Salmonella eimsbuettel</td>
<td>679</td>
<td>Pneumonia-Viral</td>
</tr>
<tr>
<td>196</td>
<td>Bovine Virus Diarrhea</td>
<td>699</td>
<td>Rabies Negative-Human Exposure</td>
</tr>
<tr>
<td>202</td>
<td>Brucellosis</td>
<td>700</td>
<td>Rabies Negative-No Human Exposure</td>
</tr>
<tr>
<td>207</td>
<td>Caged Layer Paralysis</td>
<td>701</td>
<td>Rabies Positive-Human Exposure</td>
</tr>
<tr>
<td>222</td>
<td>Coccidiosis</td>
<td>702</td>
<td>Rabies Positive-No Human Exposure</td>
</tr>
<tr>
<td>244</td>
<td>Deficiency Disease-Calcium</td>
<td>703</td>
<td>Rabies</td>
</tr>
<tr>
<td>245</td>
<td>Deficiency Disease-Magnesium</td>
<td>733</td>
<td>Toxicity-Dieldrin</td>
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<tr>
<td>247</td>
<td>Deficiency Disease-Manganese</td>
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<td>Toxicity-Drugs</td>
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<tr>
<td>302</td>
<td>Enteritis-Infectious Feline</td>
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<td>Toxicity-Ethylene Glycol</td>
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<tr>
<td>494</td>
<td>Neo-Adenoma-Mammary Gland</td>
<td>779</td>
<td>Toxocity-Garbage</td>
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<tr>
<td>496</td>
<td>Neo-Adenoma-Ovary</td>
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<td>Toxicity-Nitrate</td>
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<td>512</td>
<td>Neo-Fibroma</td>
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<td>Toxoplasmosis</td>
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<tr>
<td>513</td>
<td>Neo-Fibrosarcoma</td>
<td>826</td>
<td>Vibrionic Dysentery</td>
</tr>
<tr>
<td>536</td>
<td>Neo-Mixed Mammary Tumor</td>
<td>828</td>
<td>Virus Pig Pneumonia</td>
</tr>
<tr>
<td>551</td>
<td>Neo-Seminoma</td>
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</table>
### ANIMAL BREED CODES

<table>
<thead>
<tr>
<th>Code</th>
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<td>01</td>
<td>Holstein</td>
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<tr>
<td>02</td>
<td>Guernsey</td>
</tr>
<tr>
<td>03</td>
<td>Jersey</td>
</tr>
<tr>
<td>04</td>
<td>Ayrshire</td>
</tr>
<tr>
<td>05</td>
<td>Brown Swiss</td>
</tr>
<tr>
<td>35</td>
<td>Afghan Hound</td>
</tr>
<tr>
<td>36</td>
<td>Airdale Terrier</td>
</tr>
<tr>
<td>37</td>
<td>Basenji</td>
</tr>
<tr>
<td>38</td>
<td>Basset Hound</td>
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<tr>
<td>39</td>
<td>Beagle Hound</td>
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### SEROLOGY CODES

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<td>Anaplasmosis</td>
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<td>02</td>
<td>Brucellosis</td>
</tr>
<tr>
<td>03</td>
<td>Leptospirosis</td>
</tr>
<tr>
<td>04</td>
<td>L. pomona</td>
</tr>
<tr>
<td>05</td>
<td>L. icterohaemorrhagiae</td>
</tr>
<tr>
<td>06</td>
<td>L. hardjo</td>
</tr>
<tr>
<td>09</td>
<td>L. grippotyphosa</td>
</tr>
<tr>
<td>11</td>
<td>L. canicola</td>
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### COUNTY CODES – STATE OF IOWA

<table>
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<th>Code</th>
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</tr>
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<tbody>
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<tr>
<td>04</td>
<td>Appanoose</td>
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<tr>
<td>05</td>
<td>Audubon</td>
</tr>
<tr>
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<td>Benton</td>
</tr>
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<td>Black Hawk</td>
</tr>
<tr>
<td>08</td>
<td>Boone</td>
</tr>
<tr>
<td>09</td>
<td>Bremer</td>
</tr>
<tr>
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<td>Buchanan</td>
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### SPECIES OF ANIMAL AND/OR TYPE OF SPECIMEN

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<tr>
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</thead>
<tbody>
<tr>
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<td>Dried Milk</td>
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<tr>
<td>011</td>
<td>Fish Meal</td>
</tr>
<tr>
<td>015</td>
<td>Hay</td>
</tr>
<tr>
<td>030</td>
<td>Silage</td>
</tr>
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<td>Water</td>
</tr>
<tr>
<td>106</td>
<td>Bat</td>
</tr>
<tr>
<td>107</td>
<td>Bear</td>
</tr>
<tr>
<td>113</td>
<td>Bovine</td>
</tr>
<tr>
<td>116</td>
<td>Canine</td>
</tr>
<tr>
<td>193</td>
<td>Porcine</td>
</tr>
</tbody>
</table>
THE LABORATORY DIAGNOSIS
OF MYCOPLASMA INFECTIONS

Julius Fabricant and T. L. Barber*
Department of Avian Diseases
New York State Veterinary College, Cornell University
Ithaca, N.Y.

The laboratory diagnosis of mycoplasma infections may be divided into two distinct problems. The first and simpler problem is the diagnosis of mycoplasma infections which cause specific diseases and whose cultural and serological properties have been established. The second and more difficult problem is the isolation and identification of mycoplasma whose pathogenic potential has not been established or the search for new mycoplasma species which may be of clinical significance. The purpose of this paper is not to offer a manual of methods to the diagnostician; space and the present state of our knowledge hardly permit this, but to present and evaluate the basic technical procedures used and suggested for mycoplasma diagnosis.

I. The diagnosis of specific diseases whose mycoplasmal etiology has been established.

This is a relatively simple task because the search may often be limited to one specific mycoplasma species or its specific antibodies. This is especially true with such organisms as M. gallisepticum, M. mycoides var. mycoides, or M. agalactiae where identification is more important for epidemiological than for clinical purposes. In these cases, serological procedures are not only the most sensitive diagnostic tools but also the only methods that can be applied practically on a field scale for use in control or eradication programs. The etiologic agents of avian infectious synovitis (M. synoviae), enzootic pneumonia of pigs (M. hyopneumoniae) and M. meleagridis in turkeys may soon enter a similar stage.

A more complicated problem is posed by those mycoplasma species which have been recognized as pathogens in natural and experimental infections but whose characteristics, distribution and epidemiology are not yet clearly understood. Some of these mycoplasma may turn out to be sufficiently important to justify eradication programs while others may be considered as facultative or accidental pathogens capable of persisting as part of the “normal” microflora of the host species for long periods of time.

Thus, it can be expected with mycoplasma as with other microbial agents that all varieties and gradations of host-parasite interrelationships exist. Therefore, our use of diagnostic methods and our interpretation of their significance must also reflect our understanding of this host-parasite relationship.

a) Isolation and Identification of specific mycoplasma species

Once the etiological role and basic biological characteristics of a given pathogenic mycoplasma has been established, it is relatively easy to devise specific cultural procedures to isolate that organism from clinical material. This has now

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been done for a long series of mycoplasma.4,14,16,24,37,39,40

If the clinical material to be cultured comes from deep-seated or localized lesions, it has a high likelihood of yielding a pure culture of the particular pathogenic mycoplasma. Such material is most likely to be derived from necropsy specimens. This is apparently also true of cultures derived from mycoplasmal mastitis3,22 or arthritis31,36. On the other hand when attempts are made to isolate mycoplasma from respiratory or genital mucous membranes, a large percentage of the isolates contain more than one species of mycoplasma.2,4,9,10,15,20,24,33,42

It should be noted that the standard cultural procedures in use today make it very difficult for any organism other than another mycoplasma to contaminate mycoplasma cultures. Penicillin and thallium acetate are very effective inhibitors of bacteria and fungi while viruses are incapable of multiplication in cell-free media and are therefore effectively diluted out after a few passages. The latter statement, however, is predicated on the fact that no virus has yet been detected which is capable of growing in mycoplasma.

In addition to the problem of mixed cultures there are several other major disadvantages to mycoplasma isolation as a diagnostic tool. Isolation techniques are slow and relatively expensive. Many species of mycoplasma are difficult to isolate even for experienced research workers. The many species of mycoplasma require a variety of culture media for successful cultivation.4,5,24 No single medium will support the growth of all of our presently known laboratory cultures and some mycoplasma species have very specific and unusual cultural requirements. For example, \textit{M. synoviae} will grow only when free nicotinamide adenine dinucleotide (NAD, DPN) is added to the medium6 and \textit{M. bovigenitalium} grows poorly or not at all without added soluble desoxyribonucleic acid (DNA).13

Even after mycoplasma colonies are grown on culture media, they must still be specifically identified. Until recently such identification was usually carried out by preparing an antigen from the mycoplasma culture which could be subjected to any of a number of serological procedures with known reference antisera. This is also a slow and expensive procedure and usually requires preliminary culture purification.

Several recent studies3,10,30 have demonstrated the suitability, rapidity and practicality of immunofluorescent staining of mycoplasma colonies for their identification. In view of the extensive use of such procedures for viral diagnosis, a fluorescent microscope will probably soon be available to most diagnostic laboratories. Much technical work still remains to be done before this will be a routine diagnostic procedure but its speed and specificity are important advantages. However, the use of immunofluorescent diagnostic reagents is dependent on the purity of the antigen used to make the antibody from which the globulin is extracted. Such pure antigens are only available for our well-characterized mycoplasma species. Once specific labeled antibody is available, immunofluorescence will identify a given mycoplasma species even in a mixed culture on a primary isolation plate. Immunofluorescence is most useful when the scope of the diagnosis is limited to detection of a single mycoplasma species, or a few species. The technique becomes unwieldy and more expensive if a wide array of mycoplasma species must be identified. This disadvantage is intensified by the absence of known useful group antigens among the mycoplasma and the present paucity of information on methods for their grouping on the basis of biochemical
and biophysical characterization procedures. Some procedures for such a preliminary division of mycoplasma into valid biologic subgroups is a necessary prerequisite to the efficient use of immunofluorescence except for limited purposes.

The data available at the present time on the biochemical and biophysical characteristics of the various mycoplasma species is too scanty to allow the use of these procedures for culture identification except in a few isolated cases. Not only is such data scarce and incomplete but also much of it is inaccurate or incorrect. Some of the characterization data reported in the literature was obtained on mixed or mislabeled cultures. In most cases, an insufficient number of isolates were examined to accurately reflect the true parameters of a given mycoplasma species. Further confusion was introduced by the fact that the composition of the culture media on which the tests are performed will affect the nature of the reaction observed. Even such a simple and important characteristic as dextrose fermentation may be changed from positive to negative by minor alterations in the composition of the culture medium.

b) Diagnosis of mycoplasmal infections by serological means

As we have indicated above, once sufficient information is available and the preliminary technical difficulties have been solved, the detection of specific antibodies in the serum of infected animals is usually the most useful procedure for the diagnosis of mycoplasma infections. A wide variety of serological techniques has been used successfully for this purpose. Rapid plate or tube agglutination, hemagglutination-inhibition, direct or indirect, complement-fixation, agar gel precipitin, metabolic-inhibition and other procedures have been used. Such a serological diagnosis is most useful and most accurate in those mycoplasmal infections which produce a severe and/or generalized disease. They may be less useful for the detection of mild infections or localized infections such as bovine mycoplasmal mastitis.

It should be reemphasized that neither the detection of mycoplasmal antibodies nor the isolation of a mycoplasma culture is prima facie evidence that mycoplasma are the etiological agent of the clinical condition under study. The antibodies could be due to a previous infection unrelated to the present case while the isolation may indicate the occurrence of a carrier state and also not be the cause of the present condition.

II. The search for new pathogenic mycoplasma and the study of the relation of mycoplasmal flora to other infections.

There are three major areas where the search for new pathogenic mycoplasma has been concentrated. These are the respiratory tract, the genital tract and arthritic lesions. This search is justified for three reasons:

1. Mycoplasmal diseases or lesions have already been found in these areas.
2. Significant numbers of undiagnosed clinical lesions occur in these areas.
3. Mycoplasma species of undertermined pathogenicity have also been isolated from these areas.

It is worth commenting at this point that Chlamydia sp. should also be considered as potential pathogens in these same areas and for the same reasons.

Studies in several animal species indicate that the true clinical significance of some mycoplasma species is not as primary pathogens. Rather their importance is
due to their role in paving the way for some secondary infection. The best documented case of this type is the relationship between *M. gallisepticum* and *Escherichia coli* infections in chickens.\textsuperscript{18,19} Essentially no data has been collected as yet on possible similar relationships between the other avian respiratory mycoplasma and *E. coli*.\textsuperscript{26}

In the process of trying to isolate pathogenic mycoplasma research workers have isolated and described many species of mycoplasma from the respiratory and genital tracts of a variety of animal species. They may also be present in digestive tract but very little work has been done in these area.

The biological role of this extensive mycoplasma flora of the various mucous membranes has not yet been carefully investigated. Some may be primary pathogens, some facultative pathogens and some may play a role in assisting or hindering the multiplication or invasiveness of other microorganisms.

For all of the above reasons, a careful study of the mycoplasmal flora of our domestic animals is an important task for the veterinary microbiologist or experimental pathologist. For much of the preliminary observations and some of the primary isolates the research workers are dependent on the veterinary laboratory diagnosticians.

Once we have redefined our objectives in this manner it is obvious that the crucial first step in such studies is the isolation and identification of all of the mycoplasma species found in animals. All further work on distribution, characterization, classification, serology, pathogenicity or other biological significance cannot be done without the specific microorganisms in question.

Repeated studies in our laboratory\textsuperscript{4,5,24} have indicated the importance of using a variety of culture media for such primary isolation studies. Too much work in the past has been limited to the use of one or two culture media. Even though such an approach may yield a high percentage of positive isolations, these isolations may represent only that part of the mycoplasmal flora that grow well on such media and will have missed large numbers of organisms, and more important, certain species of mycoplasma with different cultural requirements. The time has come to disabuse ourselves of the idea that all mycoplasma are very similar. This group of organisms is intensely varied in its characteristics. We can no more expect an accurate determination of the mycoplasmal flora with a limited group of culture media than we could expect an accurate determination of the bacterial flora with the same type of limited approach.

On the other hand, it would be impractical and impossible at the present state of our knowledge of the cultural characteristics of mycoplasma to expect to be able to isolate all of the mycoplasma. In addition to the specific and unusual cultural requirements of *M. synoviae* and *M. bovis genitalium* mentioned previously, another group of mycoplasma, the T strains\textsuperscript{35}, only grow well at pH 6 and are relatively sensitive to thallium acetate. Undoubtedly, other similar cases of such specific growth requirements exist. It is impossible to predict in advance which of our as yet unrecognized mycoplasma species also have such unique cultural requirements.

In our laboratory we have arbitrarily compromised on the use of a minimum of five different culture media when investigating the occurrence of mycoplasma in a new ecological niche. Naturally the value of a given culture medium is dependent on its ability to grow the specific mycoplasma occurring in that particular niche.
Therefore, it is not surprising that we have found the choice of culture media to be used can only be determined by comparative primary isolation studies on the specific clinical material in question and will differ from one niche to another.

Such studies have shown that relatively few mycoplasma species can be readily isolated on a wide variety of culture media. We have also observed that the use of a multiple culture media increases the chance that more than one species of mycoplasma may be isolated from a given clinical specimen. The investigation and evaluation of procedures for primary isolation of mycoplasma is one of the most important tasks we must perform.

Although a wide variety of culture media have been used over the years and although it is impossible to predict which culture media will be useful in a new situation, the following minimum list of media is presented because these media have had a wide range of applicability in our experience. In each case the broth form of the medium is listed first, the agar plate form second. We have found it advisable in media evaluation to use each medium both by direct plating and by plating after three days of incubation at 37°C in the corresponding broth medium. All plates are examined after 6 days of incubation at 37°C in a candle jar with added moisture.

**BS and BA medium**\(^{16}\) – Difco Heart Infusion Broth or Agar supplemented with ten percent swine serum and bacterial inhibitors.

**II and IIP medium**\(^{17}\) – the same as BS-BA except that also supplemented with ten percent yeast extract (Chanock type).

**C and CP medium**\(^{7}\) – Difco PPLO Broth or Agar supplemented with twenty percent horse serum and ten percent yeast extract (Chanock type).

**RYE and RYEP medium**\(^{34}\) – Homemade rabbit infusion supplemented with ten percent rabbit serum and ten percent yeast extract (Chanock type).

**VFS and VFSP medium**\(^{5,40}\) – A peptic digest of beef and beef liver – made with pig stomach as the source of pepsin – this medium is never autoclaved but sterilized by filtration.

Since our experience has already shown that the basal medium, the serum supplement, the particular yeast supplement used as well as many other factors may stimulate the growth of some mycoplasma or inhibit the growth of others, it is obvious that many other possible varieties of culture media might be useful in different circumstances.

The second important problem in this phase of mycoplasma research is the purification and identification of the mycoplasma isolates. A large proportion of such isolates from mucous membranes will contain more than one species of mycoplasma. It is impossible to accurately characterize, identify and classify such cultures until they are purified. At the present time no simple dependable means exist for culture purification. The presently accepted procedure of cloning (colony picking) three time from terminal dilutions is no guarantee of culture purity. This procedure has failed many times in our hands as well as in other laboratories. It should be noted that up to this time no one has successfully initiated growth of a mycoplasma culture from a single organism.

Such a cloning procedure even when it does work, also has the disadvantage of discarding all components of culture except the most numerous component. Slightly better success has been achieved by carrying out the cloning procedure on a
series of different culture media, where some degree of selection pressure is exerted by the media or by cloning a relatively large number of colonies in the hope that some of them will be pure.

The failures of the cloning procedure are probably explained by the observations\textsuperscript{3,10} with immunofluorescence that some mycoplasma colonies may contain more than one mycoplasma species or by the fact that all of the viable mycoplasma on the plate do not form colonies.

It should be obvious, although it does not seem to have been widely recognized until recently, that the solution to the mycoplasma purification problem lay in the combined use of cloning, characterization procedures and serological procedures.

For the past years, our laboratory has been intensively engaged in the search for biochemical and biophysical procedures which could be used to differentiate mycoplasma species. When we started this work there were four major procedures which we had found useful. A recent report\textsuperscript{43} from this laboratory has greatly extended the list of useful procedures.

The original four procedures were:

1. Fermentation of Dextrose
2. Tetrazolium Reduction
3. Arginine Decarboxylation
4. Film and Spots Reaction

To these were added:

5. Fermentation of Saccharose
6. Fermentation of Mannose
7. Fermentation of Mannitol
8. Fermentation of Levulose
9. Fermentation of Cellobiose
10. Fermentation of Sorbitol
11. Fermentation of Salicin
12. Fermentation of Galactose
13. Fermentation of Xylose
14. Fermentation of Glycerin
15. Reduction of Methylene Blue
16. Growth at pH 5.5
17. Growth at pH 9.5
18. Growth at 25° C
19. Growth at 42° C
20. Growth in 1 percent Bile Salts
21. Growth in 3 percent Sodium Chloride
22. Growth in 0.2 percent Methylene Blue
23. Inhibition of growth by Kanamycin
24. Inhibition of growth by Erythromycin
25. Growth without serum

This series of characterization procedures combined with serological studies has enabled us to detect many previously unrecognized mixed cultures (indicated by the presence of aberrant characterization results) and to purify many of them through the use of differential growth patterns in procedures 16 to 25. It is our hope that by continuing the search for new characterization procedures and
evaluating their use, we will be able to devise a series of differential media to be used in a flow sheet pattern that will allow more accurate mycoplasma culture purification. We also hope that during the course of these studies, group patterns will be detected which will allow the splitting of mycoplasma into families and genera which can then be specifically diagnosed by serological typing.

This brief summary of the present status of mycoplasma diagnosis and identification should guide your decisions on the extent that you can participate as laboratory diagnosticians in the solution of these problems.
REFERENCES


A NEW ROLE FOR WILDLIFE IN DISEASE EPIZOOTIOLOGY*

Daniel O. Trainer

When considering the role that wildlife plays in the epizootiology of disease, the usual associations which come to mind are its roles as 1) a reservoir of disease such as tularemia or plague, 2) a vector of a malady such as rabies, or 3) the primary target of a disease such as botulism or leucocytozoon. Wildlife can play all of these epizootiologic parts, but another role seldom considered involves the use of wildlife populations as disease sentinels for the detection and prediction of epizootics. This will be the subject of discussion in this presentation.

The Department of Veterinary Science, University of Wisconsin, has been interested in wildlife diseases for some time and have sought primarily through the use of serologic methods to learn more about infectious diseases in wild populations. When this serologic data has been integrated with other epizootiologic information, the findings have proven to be both interesting and useful.

*From the Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin, Published as V. S. paper no. 43.
The serologic results of wild turkeys at Welder reflected the SLVE activity in human populations of the area.

All turkeys were bled during the winter period, January and February, and recorded as that year; therefore, the serologic results reported for a specific year actually indicates virus activity of the previous year(s). The turkey reactors reported in 1965 therefore, had undoubtedly been exposed during the summer of 1964. In retrospect the results of this turkey survey predicted the SLVE human outbreaks of 1965 and 1966.

A very similar situation occurred with western viral encephalitis in Alberta Canada where the disease occurred in snowshoe hares prior to an epizootic in horses. In this instance the disease in the wild host proceeded an epizootic in livestock by 6 weeks.

**California Viral Encephalitis:**

The number one big game species in North American is the white-tailed deer. To obtain information on the prevalence of specific diseases in deer numerous serologic studies have been done, (i.e., brucellosis, leptospirosis, arboviruses). The serologic results for California viral encephalitis (CVE) was of particular interest. In one survey approximately 1300 white-tailed deer sera from seven states were examined for CVE neutralizing antibodies. Thirty percent of the deer were serologic positives and the reactor rate varied from zero in some sites such as New York State and Quebec to as high as 26 percent in Wisconsin and 50 percent in Texas (Table 1). The Wisconsin deer sera were collected in 1961, 1962, 1963, and 1965. In 1961 and 1962 approximately half of the sera tested were CVE reactors while in 1963 and 1965 only 6 and 15 percent were positive. Similar annual variations were noted in Texas deer.

The largest number of CVE reactors occurred in areas in which virus was reported to be enzootic, Wisconsin and Texas. There has as yet been no association of overt disease in deer with the viruses of CVE.

As part of this same study, 46 deer sera were found to be serologic reactors to vesicular stomatitis virus (VSV) and 90 percent of these were from Texas (Table 1) an acknowledge VSV area. The 500 Texas sera were collected over a 4 year period (1963-66) and 2, 15, 2, 10 percent reacted annually. The most reactors (15%) occurred in 1964 suggesting a possible increase in VSV activity at this time. An epizootic of vesicular stomatitis occurred in cattle in Texas during 1964 which corresponds with the observed increased VSV reactor rate in deer.

These deer sera were also tested for antibodies to eastern and western viral encephalitis (Table 1). There were no reactors against eastern and 4 percent reacted against western. Most of the positives were from Texas (7%), Nebraska (62%) and Wyoming (4%), areas where western viral encephalitis might be expected to occur.

The deer sera from Quebec and their lack of serologic reactors is interesting but not totally unexpected. These serum samples were from deer on Anticosti Island, located 50 miles from the mainland. This is therefore removed from other deer and livestock and presents a unique isolated deer herd, possibly with limited exposure to virus activity.

The restriction of serologic reactors to certain viruses in appropriate geographic areas, the occurrence of reactors in high numbers in enzootic years and the absence
of reactors in interepizootic years, reactors to only one antigen, the complete lack of reactors in an isolated island population, all add credence to the fact that the methods used were detecting antibody against the specific antigens.

From these results it appears that white-tailed deer are sensitive indicators of the presence of many arbovirus infections. Because of their large populations (20 million), the ubiquitous distribution (Panama to Canada and coast to coast), non-migratory behavior, the ease and accuracy of sexing and aging animals, and the fact that 2.5 million deer are harvested annually by hunters thus providing a ready supply of sera, this wild species could serve as a valuable indicator species for the activities of viruses — a wild sentinel.

**Leptospirosis:**

Disease prevalence in a wild species is not necessarily related to disease prevalence in non-wild species. Serologic studies of leptospirosis in white-tailed deer and cattle in Wisconsin illustrated that there was no direct relationship between the reactor rate in one population compared to the other. Of 1256 deer sera tested against 13 *Leptospira* serotypes from three distinct geographic areas of Wisconsin, 25 percent were reactors and the predominate serotype was *L. pomona* (78 percent). When deer reactor rates of the respective study areas were compared with similar information for cattle, a direct relationship was not apparent (Table 2). For example, in the northern study area (c) 22 percent of the deer were positive, while none of the cattle reacted. In deer, the prevalence of leptospirosis appeared to be related to population density of deer; in cattle, a number of factors including population density and herd management were important. Studies in Canada have reported evidence of leptospirosis in deer and moose hundreds of miles from any livestock activity.

Despite the fact that lepto-reactors are numerous in deer, their detection and reactor rate does not necessarily reflect the status of leptospirosis in livestock.

**Conclusions:**

From the examples cited it would appear that serologic studies of wildlife can under the proper circumstances be utilized to monitor and even sometimes predict human and livestock disease outbreaks. To utilize wild populations for this "sentinel" duty certain specific conditions must exist:

The wild population must 1) have a known limited home range so that the area being monitored can be defined, migratory population would not be appropriate; 2) be present in good numbers and readily accessible so that test sera can be received easily periodically, such as wild turkeys and deer; 3) contain individuals which are easily bled, aged, and sexed; 4) be susceptible and respond serologically, yet not be adversely affected by the disease under study.

The disease to be monitored must 1) be readily transmitted between the selected wild and domestic populations; 2) produce a non-lethal disease in the sentinel host; 3) stimulate a detectable serologic response.

When the above predisposing factors are properly integrated, such as with the white-tailed deer and California viral encephalitis, a "natural" monitoring system can be in effect on a local, national, or even continental basis. The potential for such a system is unlimited and could be expanded to include other infectious as well as non-infectious maladies, such as pesticides, radio-activity, etc.
Summary:
Wildlife are usually considered as vectors, reservoirs, or primary targets of infectious disease. Another role which they can play in epizootiology involves the use of wild populations as a disease sentinel for the detection and prediction of epizootics. Such use was demonstrated with the wild turkey and St. Louis viral encephalitis in Texas, the snowshoe hare and western viral encephalitis in Canada, and the white-tailed deer and California viral encephalitis in North America. The limitations and criteria which are involved in the utilization of wild populations for “sentinel” duty are discussed.
LITERATURE CITED


NEW ROLE FOR WILDLIFE IN DISEASE EPIZOOTIOLOGY 587

TABLE 1.
SEROLOGIC RESULTS OF WHITE-TAILED DEER SERA FROM 7 STATES OR PROVINCES FOR SELECTED ARBOVIRUSES.

<table>
<thead>
<tr>
<th>Serum Source</th>
<th>No. Sera</th>
<th>CVE</th>
<th>VSV</th>
<th>WVS</th>
<th>EVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quebec</td>
<td>103</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wyoming</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>New York</td>
<td>122</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Iowa</td>
<td>28</td>
<td>21</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nebraska</td>
<td>8</td>
<td>75</td>
<td>0</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>512</td>
<td>26</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Texas</td>
<td>518</td>
<td>50</td>
<td>8</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,314</td>
<td>30</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Reactors neutralized $10^{1.5}$ to $10^{2.5}$ ID$_{50}$ of specific antigen in HeLa Cells in a metabolic inhibition test.

TABLE 2.
PREVALENCE OF LEPTOSPIROSIS REACTORS IN WHITE-TAILED DEER AND CATTLE FROM THREE WISCONSIN STUDY AREAS.

<table>
<thead>
<tr>
<th>Study Area</th>
<th>Population per sq. mile</th>
<th>Percent reactors</th>
<th>Deer</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deer</td>
<td>Cattle</td>
<td>Deer</td>
<td>Cattle</td>
</tr>
<tr>
<td>A (south)</td>
<td>5-10</td>
<td>106</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>B (central)</td>
<td>30-40</td>
<td>39</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>C (north)</td>
<td>20-30</td>
<td>3</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 1. The prevalence of St. Louis Encephalitis serologic reactors in a wild turkey population in south Texas (1963-67).
INTRODUCTION

Bacterial colonies surrounded by a wide zone of complete hemolysis quickly attract the attention of the diagnostic microbiologist. Conversely, less hemolytic colonies are less conspicuous and much easier to ignore. This is true especially for alpha hemolytic Streptococcus spp. that may characteristically and frequently be encountered as supposedly non-pathogenic inhabitants of many surfaces of the animal body. Alpha hemolytic streptococci of the Viridans or Enterococcus Groups in mixed culture with E. coli and/or micrococci are customarily disregarded in a quick search for bacterial pathogens on culture plates.

It is only when alpha hemolytic Streptococcus spp. are repeatedly isolated in pure culture from the same tissue source and from the same recognizable disease syndrome that they begin to be afforded much attention. Thus attempts have recently been made to characterize the alpha hemolytic streptococci recovered from pneumonic porcine lungs and to perhaps determine their possible pathogenic significance.

In his review of the literature dealing with swine pneumonia, L'Ecuyer has stated that many bacterial species have been incriminated as primary agents of swine pneumonia, but that only a few have been confirmed experimentally. Alpha hemolytic streptococci are not in that confirmed group. In his rather complete survey of bacterial species isolated from normal and pneumonic swine lung specimens, L'Ecuyer counted and partially characterized 17 biochemically distinct strains of alpha hemolytic streptococci. None of these appears to be identical with the recent isolates described in this paper, however. He, like others preceding him, did not attempt to reproduce pneumonias in experimental pigs with any of the alpha hemolytic streptococcus isolates.

MATERIALS AND METHODS

Source of field isolates: Swine or swine tissues submitted to the Iowa Veterinary Medical Diagnostic Laboratory were the source of all field specimens. If, on routine bacteriological examination, alpha hemolytic streptococci were recovered in pure culture from lungs, heart's blood or joint fluid and were from a field case having typical disease history, isolates were preserved for further study.
Preservation of cultures: Typical isolates survived poorly in serum broth cultures. They tended to remain viable for six weeks or longer on the surface of bovine blood agar slants stored in the dark at room temperature. Lyophilization or freezing might be advisable as a means of preserving viability of cultures for longer periods.

Isolation procedure: Five percent citrated bovine blood agar plates were a satisfactory medium for isolation. Initial growth was enhanced markedly by anaerobic incubation in sealed jars employing disposable GasPak® carbon dioxide and hydrogen generators. Micrococcus spp. nurse colonies were streaked on primary plates as an aid in the detection of Hemophilus spp., if present, but they failed to enhance the growth of the alpha hemolytic streptococci.

Isolated colonies were routinely subcultured in tryptose broth containing 10 percent by volume sterile bovine serum. After 24 hours' growth and if pure, this culture was used as inoculum for biochemical test media, antibiotic sensitivity test plates and blood agar slants.

Biochemical tests: Standard carbohydrate fermentation tests were read at the end of 24 hours' incubation in the following medium:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>5.0 g.</td>
</tr>
<tr>
<td>Tryptose, Bacto®</td>
<td>7.5 g.</td>
</tr>
<tr>
<td>Proteose peptone No. 3, Bacto</td>
<td>7.5 g.</td>
</tr>
<tr>
<td>Beef extract, Bacto</td>
<td>3.0 g.</td>
</tr>
<tr>
<td>Appropriate carbohydrate</td>
<td>5.0 g.</td>
</tr>
<tr>
<td>Andrade's indicator</td>
<td>10.0 ml.</td>
</tr>
<tr>
<td>Distilled, deionized water</td>
<td>q.s. 1,000.0 ml.</td>
</tr>
<tr>
<td>Adjusted to pH 7.4 prior to sterilization</td>
<td></td>
</tr>
</tbody>
</table>

Other differential tests: Details for the preparation of other test media and the performance of tests may be obtained by consulting references in appropriate sections of Bergey's Manual.5

RESULTS

Incidence: From among 1,987 porcine accessions at the Iowa Veterinary Medical Diagnostic Laboratory between July 1, 1968 and April 30, 1969, there have been 62 cases where pneumonia has been diagnosed as the principal disease condition. In 24 of these cases alpha hemolytic streptococci have been isolated as the only bacterial pathogen. Although isolations have mostly been recorded from pneumonic lung lesions, recovery of the same agent in pure culture from heart's blood, joint fluid and other parenchymatous organs has frequently indicated that alpha hemolytic streptococci may also cause acute septicemia in young pigs.

Typical case history: Isolations of alpha hemolytic streptococci have been most frequent in field cases of the following description:

- Nursing pigs 2 to 3 weeks of age
- Sudden onset of disease
- Acute deaths of 2 to 4 pigs per litter
- Many litters affected

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5Baltimore Biological Corporation
bDifco Laboratories, Inc.
Swelling of joints and stiffness common
Anemia frequent

*Post mortem observations:*
- Severe fibrinopurulent bronchopneumonia
- Hematogenous distribution of lesions
- Possibly superimposed on lesions associated with *M. hyopneumoniae* infection (VPP)
- Pericarditis
- Endocarditis
- Arthritis
- Catarrhal enteritis

*Histopathological observations:* Microscopic lesions thus far observed are characteristic of acute pneumonia of bacterial etiology. No specific lesions can be cited which are pathognomonic for this syndrome in lung tissue sections.

*Colony morphology and hemolysis:* Alpha hemolytic streptococci are usually recovered in pure culture from typical cases. On bovine blood agar plates, colonies are rather flattened and grey with a narrow zone of incomplete greenish hemolysis that becomes more apparent with age. Growth is definitely enhanced by anaerobic incubation after which the colonies appear comparatively larger, more mucoid and hemolytic. At first glance, alpha hemolytic streptococcus colonies might be confused with those of *Pasteurella multocida* except for their hemolytic property. The distinction is more striking when anaerobic growth conditions are employed, since anaerobiosis enhances streptococcal growth and inhibits that of *Pasteurella multocida*.

*Cell morphology and staining:* Typical isolates are Gram positive cocci which grow in pairs or very short chains. Cells tend to be somewhat elongate, but not distinctly lancet shaped or pointed as with the diplococci. A capsule is detectable if India ink mounts are prepared from fresh isolates. Growth in broth cultures is uniformly turbid and does not sediment as does that of many long-chain streptococci.

*Bile solubility and optochin sensitivity:* *Diplococcus pneumoniae* isolates are often tentatively identified by bile solubility tests and zones of clearing on blood agar plates surrounding optochin discs. Although alpha hemolytic streptococci isolated from swine pneumonia cases may superficially resemble diplococci in many other respects, all streptococcus cultures thus far tested have been uniformly insoluble when washed cells are suspended in 10 percent oxgall solution. Neither are there zones of clearing surrounding optochin discs on test plates.

*Carbohydrate fermentation reactions:* Alpha hemolytic *Streptococcus spp.* isolated in pure culture from porcine lungs fall into a definite biochemical grouping. They uniformly ferment sucrose, salicin, raffinose, inulin, trehalose, lactose and dextrose without gas production. Mannitol and sorbitol are not fermented in 24 hours' of incubation. Other alpha hemolytic streptococci isolated from mucous membranes or the gastrointestinal tract of swine differ significantly in biochemical properties. Comparative biochemical test results also showing other potential pathogens are shown in Table I.

*Further biochemical characterization:* These porcine streptococcal isolates were subjected to additional biochemical tests as described in Bergey's Manual.5
<table>
<thead>
<tr>
<th>Bacterial species:</th>
<th>Alpha hemolytic streptococci of porcine lung</th>
<th><em>Strep. pyogenes</em></th>
<th><em>Strep. equisimilis</em></th>
<th><em>Strep. zooepidemicus</em></th>
<th><em>Strep. Lancefield Group E.</em></th>
<th>Diplococcus pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litmus Milk acid</td>
<td>+a</td>
<td>+</td>
<td>v_d</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>coagulation</td>
<td>b</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>reduction</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>0^c</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>V</td>
<td>0</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dextrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bile solubility</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

^aPositive reaction (+)
^bNegative reaction (-)
^cResult unavailable (0)
^dVariable (V)
Test results were identical for all isolates that had not already been differentiated from this group on the basis of initial fermentation reactions. The results in Table II clearly indicate that these streptococci may best be identified as members of the Pyogenic Group which also contains most other *Streptococcus* spp. pathogenic for animals and man. They therefore have been further differentiated from the Viridans, Enterococcus or Lactic Groups which are known to contain most alpha hemolytic streptococcus species that are seldom regarded as pathogens.

From these studies however, it has not yet been possible to designate a species name for this group of bacterial isolates.

**Antibiotic sensitivity:** The alpha hemolytic streptococci isolated from porcine pneumatic lungs were invariably susceptible to the action of furacin, chloromycetin, erythromycin, penicillin and terramycin based on the results of *in vitro* sensitivity tests. They were less likely to be inhibited by streptomycin, neomycin or polymyxin B. It was difficult to assess sulfonamide sensitivity due to poor growth of the organisms on Mueller-Hinton medium.

**Pathogenicity:** Young cultures of fresh field isolates kill mice in less than 24 hours following intraperitoneal injection of as little as a 0.1 ml. dose. Arrangements are in progress for attempts to reproduce the characteristic disease syndrome experimentally in 2-week-old specific-pathogen-free pigs.

**TABLE II.**
Characteristics employed for grouping streptococci

<table>
<thead>
<tr>
<th>Group designation</th>
<th>Unknown porcine isolate</th>
<th>Pyogenic</th>
<th>Viridans</th>
<th>Enterococcus</th>
<th>Lactic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.5% NaCl</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>0.1% Methylene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blue Milk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 9.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>45°C C.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>100°C C.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decarboxylation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sodium hippurate</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>Ammonia from</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>V</td>
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<td>Arginine</td>
<td>+</td>
<td>+</td>
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<td>Mucoid growth</td>
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<td>Hemolysis</td>
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*a*Negative reaction (-); *b*Positive reaction (+); *c*Certain species within the group are positive. (V)
SUMMARY AND CONCLUSIONS

From repeated field cases of acute pneumonia and septicemia in 2 to 3-week-old pigs in Iowa, pure cultures of an as yet unidentified alpha hemolytic *Streptococcus sp.* have routinely been isolated. Recoveries have been most frequent from lungs, heart’s blood and joint fluids of affected pigs. Clinical signs typical of this syndrome, gross and histopathological lesions and a description of the known properties of these streptococcus isolates are reported herein.

Even if attempts to reproduce this syndrome experimentally using pure cultures of field isolates should at first prove unsuccessful, their mere recovery in pure culture in repeated cases of swine respiratory disease suggests that they may at least be highly significant secondary bacterial pathogens associated with primary virus or mycoplasma infections.

Veterinary laboratory diagnosticians should be aware of the existence of these potential bacterial pathogens, and the microbiologist should make attempts to differentiate these alpha hemolytic streptococci from those commonly encountered *Viridans* and *Enterococcus* Groups. Future research efforts must establish the possible interrelationships between the alpha hemolytic streptococci and the virus and mycoplasma swine respiratory pathogens.
REFERENCES


ACKNOWLEDGEMENTS

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INTRODUCTION

Veterinarians and livestock owners are becoming increasingly concerned with the problem of chronically coughing feedlot cattle. Though death losses are not directly attributed to the condition, the high morbidity within an affected feedlot results in poor performance of beef animals and must represent a considerable economic loss.

Many of you gentlemen here today have seen these herds, examined the animals, and listened to the owner's lament. The herd histories usually contain only one common denominator – the presence of a continual, deep hacking, nonproductive, cough in the majority of the animals in a given feedlot. Other common factors are lacking, e.g.:

1. The animals may be home raised calves or native cattle or they may have been shipped in as feeder cattle.
2. If purchased as feeders, they may have suffered from IBR and/or shipping fever or they may never have been "noticeably" ill, according to the owner.
3. They may or may not have been vaccinated against IBR, BVD, and PI3 or any combination thereof, with or without various Pasteurella spp. bacterins.
4. Heifers and steers seem to be equally affected.
5. Animals ranging in size from 300 to over 900 lbs. can be affected.

Up to now you have heard nothing new, but rather a review of a problem confronting our profession daily. We feel that we have made some observations of tracheal lesions, in our laboratory, that are new, and ask that you help us and our profession by also looking for the tracheal lesions we have seen. The lesions represent the first real clue as to what the problem may be.

MATERIALS AND METHODS

We initially followed a group of chronically coughing cattle through a slaughter plant and observed a high incidence of tracheas with hyperplastic changes within the respiratory mucosa. We have since observed the same lesions within the tracheas of specimens submitted to the Iowa State University Veterinary Diagnostic Laboratory.
POLYPOID TRACHEITIS

RESULTS
Grossly the lesions are seen as a diffuse hyperemia within the tracheal mucosa; often a thin layer of mucopurulent exudate is present. These findings are quite common in any animal suffering from respiratory distress. The hyperemia may extend from the posterior larynx to the bifurcation of the major bronchi or be mottled in distribution. Often the hyperemia is more pronounced in the posterior 1/3 of the trachea.

In the fresh specimen, one can ascertain a less-red area away from the margins of the hyperemia and if this region is observed carefully, under natural or artificial reflected light, at an oblique angle, it will appear rough. Closer observation of the mucosa with a 20-power hand lens (in the field) or under a dissecting scope (in the laboratory) will reveal a “cobblestoning” of the mucosa. As one moves from the margin of the lesions towards the center, the hyperplastic change is more pronounced and finger-like projections or polyps can be seen.

Histopathological examination of routinely stained hematoxylin and eosin sections will confirm the gross observations. Sections taken at the extreme margin of the lesions will contain an increased number of mitotic figures within the respiratory epithelium and the submucosa will be richly infiltrated with leukocytes; mostly lymphocytes and plasma cells. Neutrophils are only seen where there has been antemortem loss of respiratory epithelium.

Sections taken just inside the margin of the lesions will contain a hyperplastic mucosa forming folds and rolling or irregular in appearance. The cilia of some of the pseudostratified columnar epithelial cells will have been lost and the submucosa will, as before, be heavily infiltrated with lymphocytes and plasma cells.

Those sections towards the center of the lesions will contain the most dramatic pathological alterations. The area represents the older or more chronically affected area of inflammation, in my opinion, and the respiratory epithelium in this area is characterized by polyp formation, loss of cilia, and squamous metaplasia. Submucosal infiltration of plasma cells and lymphocytes is still dense in this location.

If we may have the lights turned out now we can look at some photomicrographs together that exemplify these changes.

*Pasteurella multocida* has been isolated in pure culture from swabs of tracheal exudate by Doctor Brown and his microbiologists. No other bacterial or viral pathogens have been isolated from the tracheas to date.

No correlation between IBR, BVD, and PI3 antibody titers and the occurrence of the condition has been found at this time.

SUMMARY
We have seen fit to call this condition “polypoid tracheitis”. Polypoid tracheitis has now been observed in our laboratory in association with pneumonic pasteurellosis, where the pneumonia was the primary feedlot malady. It has been observed in association with one case of pulmonary adenomatosis. It has been most frequently seen in the tracheas of feedlot cattle suffering from primarily a chronic cough.

We believe the inflammatory reactions observed are indicative of a chronic bacterial infection, but are suspicious of a viral agent as a predisposing factor. The
etiology and pathogenesis of the condition remains to be established.

I will conclude by saying that hyperplastic lesions within the tracheas of man, the dog, and the cat have been reported and are of unknown etiology in the dog and cat. Our physician colleagues have described hyperplastic and metaplastic changes within the tracheal and bronchial epithelium of heavy smokers, and the squamous metaplasia observed by them is considered the intermediate phase between the hyperplasia of chronic irritation and the neoplasia of respiratory carcinoma.
74th ANNUAL MEETING
October 18-23, 1970
HOTEL WARWICK
Philadelphia, Pennsylvania

75th ANNUAL MEETING
October 24-29, 1971
SKIRVIN HOTEL
Oklahoma City, Oklahoma

76th ANNUAL MEETING
November 5-10, 1972
AMERICANA OF BAL HARBOUR
Miami Beach, Florida