PROCEEDINGS

SEVENTIETH

ANNUAL MEETING

of the

UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

STATLER-HILTON HOTEL
Buffalo, New York
October 10, 11, 12, 13, 14, 1966
PROCEEDINGS
SEVENTIETH
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

STATLER-HILTON HOTEL
Buffalo, New York
October 10, 11, 12, 13, 14, 1966
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<td>J. W. Walker</td>
<td>Hyattsville, Maryland</td>
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## COMMITTEE ON PUBLIC HEALTH AND RADIOLOGICAL Fallout

R. H. Huffaker, Chairman, Atlanta, Georgia

<table>
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<tr>
<th>Name</th>
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<tr>
<td>R. Fagan</td>
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<td>N. L. Meyer</td>
<td>Washington, D.C.</td>
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<td>A. B. Park</td>
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<td>I. M. Saturen</td>
<td>Ames, Iowa</td>
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<td>J. H. Steele</td>
<td>Atlanta, Georgia</td>
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<td>E. E. Wedman</td>
<td>Ames, Iowa</td>
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<td>R. D. Wenger</td>
<td>Alexandria, Virginia</td>
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<td>A. H. Wolff</td>
<td>Washington, D.C.</td>
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## COMMITTEE ON PUBLIC RELATIONS AND LOCAL ARRANGEMENTS

L. N. Butler, Co-chairman, Phoenix, Arizona

<table>
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<th>Name</th>
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<tr>
<td>M. Bay</td>
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<td>R. A. Hendershott</td>
<td>Trenton, New Jersey</td>
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<td>H. H. Hodges</td>
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<td>E. R. Mackery</td>
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<td>N. Powers</td>
<td>Lake Luzerne, New York</td>
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<td>N. Roakey</td>
<td>Mesa, Arizona</td>
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<tr>
<td>W. Strieber</td>
<td>Hyattsville, Maryland</td>
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R. T. Hanson, Madison, Wisconsin  E. C. Sharman, Hyattsville, Maryland
<table>
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<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tr>
<td>1. Sept. 27-28, 1897**</td>
<td>Fort Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Forth Worth, Texas</td>
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<tr>
<td>2. Oct. 11-12, 1898</td>
<td>Omaha, Nebraska</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Taylor Riddle, Kansas</td>
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<tr>
<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, New York</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
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<tr>
<td>26. Dec. 6-7-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Crewe, Bismarck, N. Dak.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
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<tr>
<td>27. Dec. 5-6-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. G. Peary, Helena, Montana</td>
<td>*Dr. O. E. Dyson, Kansas City, Mo.</td>
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<tr>
<td>31. Nov. 30 - Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>*Dr. L. Van Es, Lincoln, Nebraska</td>
<td>*Dr. O. E. Dyson, Wichita, Kansas</td>
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<tr>
<td>32. Dec. 5-6-7, 1928</td>
<td>Chicago, Ill.</td>
<td>*Dr. C. A. Cary, Auburn, Alabama</td>
<td>*Dr. O. E. Dyson, Wichita, Kansas</td>
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<tr>
<td>33. Dec. 4-5-6, 1929</td>
<td>Chicago, Ill.</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
<td>*Dr. O. E. Dyson, Wichita, Kansas</td>
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<td>Date</td>
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<tr>
<td>Dec. 5-6-7, 1934</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
<td>Dec. 6-7-8, 1939</td>
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<tr>
<td>Dec. 4-5-6, 1935</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N. Y.</td>
<td>Dec. 5-6-7, 1940</td>
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<tr>
<td>Nov. 1-2-3, 1950</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. C. Green, Charleston, W. Va.</td>
<td>Nov. 4-5-6, 1958</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
R. A. Hendershott

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 of research and diagnostic procedures developed and improved upon during the past year.

We thank Thee for a very fruitful year and implore Thy guidance and Blessing on our future endeavors.

Grant that what we do here during our Seventieth Convention will be found pleasing to Thee and of value to our fellow-man to the end that we may assist in the production of wholesome and disease-free animal products to sustain our expanding population.

We beseech Thee to bless all those charged with the authority and responsibility at all levels of Government. Enlighten and guide them to the end that Thy will be done.

Amen
President Campbell, Distinguished Guests, Ladies and Gentlemen:

It is now time to pay tribute to those members of the United States Livestock Association who have passed on since our last meeting.

In our fast moving world of the space age, society, in general, tends to lose respect of individuality.

It is then most fitting that we take time, and we hope sincerely that this Association continues to pay homage to its departed members.

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

Their deeds, feats, contributions and endeavors speak for themselves.

A poet once philosophied:

"Golden Lads and Lassies Must,  
As Chimney Sweepers Come to Dust."

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

R. S. ARMSTRONG - M.S.U. - 1926; died July 25, 1966 after serving with the B.A.I. since 1929 in the State of New Jersey.

C. HERMAN BECKMAN - I.S.U. - 1920; died October 15, 1965 at the age of 68. Dr. Beckman practiced in St. Louis for 35 years, and was a past-President of the St. Louis Veterinary Medical Association.

FLOYD E. HULL - K.S.U. - 1925; died in Lexington, Kentucky on November 16, 1965. Dr. Hull had served on the University of Kentucky faculty from 1925 to 1961. He retired as head of the Department of Veterinary Science. Dr. Hull had served as past-President of the Kentucky Veterinary Medical Association.

A. L. TELLEJOHN - K.S.U. - 1936; died January 2, 1966 at Takoma Park, Maryland. Dr. Tellejohn was in the U.S.D.A. for 29 years, and was Senior Staff Veterinarian for Technical Analysis of Veterinary Biologics Division.

EDDELL C. JONES - K.S.U. - 1916; died December 9, 1965 in Lincoln, Nebraska at the age of 73. Dr. Jones was retired Chairman of the Board of Norden Laboratories. He had served as President and General Manager of the Platte Valley Serum Company until its merger with Norden Laboratories in 1934. Dr. Jones was a past-President of the Nebraska Veterinary Medical Association.
DAVID ZAITZ - killed in an automobile accident June 28, 1966 in Elgin, Illinois. Dr. Zaitz was a cattle dealer in New Jersey and was a faithful member of this Association.

JACOB TRAUM - Cornell - 1905; died August 31, 1966. Dr. Traum devoted a lifetime to research. An authority on many subjects, discovered *Brucella suis*, he authored many papers and was a real contributor to this Association.

RICHARD E. SHOPE, M.D. - died October 2, 1966 as a result of cancer - a renowned virologist and animal disease worker. Associated with the Rockefeller Institute of Medical Research. Dr. Shope gained worldwide recognition for research on Influenza and Hog Cholera. He was an honorary D.V.M. and life long member of this Association.

F. L. HERCHENROEDER - died October 9, 1966. He was a U.S.D.A. employee in the former Inspection and Quarantine Division. He was formerly in charge of the New York Area and for a number of years was in charge of the Quarantine Import Station in Athenia, N. J. He was formerly president of the National Association of Federal Veterinarians.

May we bow our heads for a moment of silent prayer. Amen.

It is impossible for us the living to properly evaluate the life and work of these men. We would need volumes to even abstract the many contributions to the science of disease control, to the livestock and poultry industries, to public health, to research, to education and related fields of endeavor.

These contributions have in no small way contributed to the overall agri-business effort in providing bountiful amounts of animal protein for this nation's food supply and food reserve. These contributions have provided adequate consumer protection in providing services assuring safe, wholesome products.

We all feel a deep sense of personal loss for these departed colleagues, but let us be thankful that we had the opportunity of knowing and working with them. Let us pledge ourselves to carry on and help complete the links of the chain, in the ever moving progress in our chosen field.
WELCOME TO BUFFALO

George C. Poppensiek*

It is a distinct privilege indeed to represent my New York State colleagues in extending a special word of greeting to those of you who have come to this meeting from other states and from other countries. It has been 65 years since the United States Livestock Sanitary Association last met in New York. Once before, in 1901, Buffalo was host to this distinguished Association, so our word of welcome is even more than a cordial greeting; it is an enthusiastic "welcome back!"

We spread before you the "land of the Mohicans," one of the strong tribes of Indians who spoke the Algonkian language; a tribe given special historical eminence by James Fenimore Cooper in his classical book "The Last of the Mohicans."

We also welcome you to the land of Hiawatha, the legendary architect of the Iroquois confederacy. The fierce nations of the Cayugas, Senecas, Mohawks, Oneidas, Onondagas and later Tuscaroras, banded together, and for many years held the balance of power between the French and English in the early settlement of the North Atlantic region; particularly New York State. Some of our lakes and counties bear their names. Other independent tribes, like the Eries and the Hurons, for whom two of the Great Lakes were named, were annihilated by the Iroquois.

The early history of New York State was quite simple; it is a story about the beaver. French fur traders dealt with the Indians, and both buyer and seller found the early trading posts to be lucrative. This raised the eyebrows of other European nationals, who sent fur traders to the wildernesses of the "new" continent west of the Old World.

When Henry Hudson sailed up the river that eventually was to bear his name, searching for the northwest passage to India, he astutely envisioned glittering guilders in the beaver pelt trade, and passed the word back home. It was not long before Peter Minuit was sent from Holland to negotiate the purchase of Manhattan Island from the Indian Chief Manhasset. We recall so well that the shrewd Hollander was indeed a wily businessman, for he purchased the island with a few bolts of brightly colored cloth, some beads and other trinkets having a market value of 60 guilders; approximately 24 American dollars.

New York has not always been New York. The new colony established on Manhattan Island by Peter Minuit was named "New Amsterdam," and this became a matter of interest to the British who also had plans for land acquisition at this point in the history of the western continent. The Dutch were aware of this. As a matter of fact, they erected a protective wall across Manhattan Island at the northern boundary of New Amsterdam, and this wall formed the basis for what is known today as "Wall Street."

*Dean, The New York State Veterinary College at Cornell University.
King Charles II of England ordered one of his military men, Richard Nicholls, to sail westward and take possession of New Amsterdam. This feat was accomplished without any resistance because the settlers were disgruntled about the dictatorial methods of Peter Stuyvesant, their peg-legged despotic governor. As soon as Charles was in possession of New Amsterdam and its environs, he gave the land to his brother, James, the Duke of York and Albany. Subsequently, the name of the territory was changed from New Amsterdam to New York; and Fort Orange, a Dutch trading post in the wilderness 150 miles up the Hudson River from New Amsterdam, was renamed Albany.

Buffalo has not always been Buffalo. The Dutch were tenacious about having an area on the new continent named for their most beloved city in the Netherlands. Joseph Ellicott, a surveyor for the Holland Land Company, named the territory currently occupied by the city of Buffalo, "New Amsterdam." In the skirmishes of conquest it was later named Clarence and in 1810 the area now occupied by the city was named Buffalo in recognition of a section of land called "Buffalo Creek" by the Indians. I am happy to report to you in behalf of the Dutch people that today New York does have its Amsterdam, on the Mohawk River, near Albany.

New York is known the world over as an industrial state, but it is also an agricultural state. Notably it ranks third in the nation as a dairy state. The dairy industry had somewhat of an unsavory beginning, however, Prior to the Civil War, milk drunk in urban areas was produced in the immediate outskirts of the growing cities. This was quite true for New York City. Our historians tell us that barns were attached to local distilleries and cattle kept in filthy condition in these barns were fed nothing more than 30 or 40 gallons of hot mash each day. They produced a blue, watery, insipid, unhealthy secretion, to which dairymen added chalk, sugar, flour, starch and coloring matter to conceal the water that they also added in generous amounts. Physicians kept a running battle against the sale of this kind of "distillery milk," blaming it largely for the high infant mortality rate. It is said that only one of every two children raised in the area reached five years of age.

With the advent of the railroad, farmers were able to send fresh milk to the cities and the dairy industry grew to the point where today 1,400,000 good New York State dairy cattle produce about 10 billion pounds of wholesome milk per year.

One commercial farmer in New York State today produces as much as five did when this Association last met in New York. Since the turn of the century, the number of commercial farmers has declined nearly 75 percent, but agricultural production has increased more than 25 percent during this period. While farm land and commercial farms have both declined about 50 percent, the average commercial farm is nearly twice as large as it was in 1900. Today only two percent of the State's labor force is in commercial farming, but one-third of the total working force is engaged in work related to agri-business. The dairy and livestock industries provide more than 60 percent of the State's gross farm income, representing better than a half billion dollars per year.
Like all other States, New York has many points of attraction in which its citizens take great pride. During the meeting of this Association many of you will have seen Niagara Falls. Some of you have seen our rich farm land, and perhaps you have visited some of the scenic parks and have enjoyed the rustic beauty of our lakes and woodlands. Others of you may have felt the excitement of our cities; have walked along Broadway and visited such cultural focal points as the Lincoln Center for the Performing Arts. But perhaps the most meaningful of all the landmarks, the one that has had a stirring impact upon the lives of so many, is the Statue of Liberty on Bedloe's Island, at the entrance to the New York harbor. Its pedestal bears a simple but eloquent inscription, written by Emma Lazarus, who knew something of the despair and oppression of rejected people, particularly from other lands. Its message is simple:

". . .Give me your tired, your poor,
Your huddled masses yearning to breathe free,
The wretched refuse of your teeming shore;
Send these, the homeless, tempest-tossed, to me,
I lift my lamp beside the golden door!"

That lamp that lights the harbor still glows, and the inscription is still the voice of America.

We hope that your stay in New York State will be both profitable and pleasant, and that you will enjoy your time here with us sufficiently so that you will want to come back. We are happy to have you.
WELCOME TO NEW YORK STATE

D. J. Wickham
Albany, New York

Here I am to welcome you after you have already been here four or five days but I have looked forward to dropping in and saying 'hello' to you folks ever since I learned that you were to meet in New York State. I am just sorry that my schedule kept me from making it earlier in the week.

It is obvious from the program that this is a working organization that keeps its nose to the grindstone, but I am glad to see that you did take a break a day or two ago for a visit to Niagara Falls. I hope you all enjoyed it.

I came to the Department of Agriculture and Markets eight years ago from a farm over along Seneca Lake (about a hundred miles east of here)—This is one of those long lakes you see on the map of western New York known hereabouts as the Finger Lakes. Over there we produce cherries and grapes in abundance but have little livestock.

This background did little to prepare me for the many activities I found going on in our Division of Animal Industry. Of course, I knew, through other connections in the field of agriculture, a little about the Tuberculosis Eradication program and brucellosis and calf vaccination. I also knew you people were doing a job on pullorum disease. But I did find a lot more going on in connection with animal disease control than I had realized.

Now I am not going to say I got to be an expert on all these things but those boys over in Animal Industry gave me as much of an education as they thought I could take. I soon got on a sort of speaking acquaintanceship with such things as anaplasmosis and hog cholera and swamp fever to name but a few.

It was at this time that I first learned about the United States Livestock Sanitary Association and the way it works to translate research progress into the practical control of disease out on the farms. I learned too that your objectives went a long way beyond mere control—that your aim was to wipe these diseases out completely—to eradicate them in other words.

I think this is good because we all know that disease can cancel out a lot of the progress made by the genetecists and the nutritionists and those working to improve the livestock industry and bolster our food supply.

And speaking of food and production—I remember the days back 25 or 30 years ago when—and even a little later than that—when we heard all that talk about agricultural surpluses. What were we going to do with all that wheat and corn. Another one of prime interest in the northeast was milk. There seemed to be more of it than people could use.

Well, you don't hear much of that talk today. I've heard it said that what we seem to have now is a shortage of surpluses. Certainly production
and demand for most agricultural commodities are much more in balance. A rising population can gradually tip the scales even farther.

The work your group is doing to cut the losses from disease will be of increasing importance as time goes by. I wish you every success in your deliberations.

I hope some of you will have the opportunity to see more of New York State after your meeting is over. In addition to our agricultural activities there is much in the way of scenic attraction. Whether or not your schedule will permit a more extended visit we do want to welcome you to New York and hope you will come back again soon.
RESPONSE TO WELCOME
H. G. Wixom, D.V.M.
Sacramento, California

Dean Poppensiek, I am honored to be privileged to speak on behalf of my fellow members of the United States Livestock Sanitary Association and to thank you for your warm words of welcome and for the graciousness in which we have been received in New York State and in Buffalo particularly.

You have made us feel very much at home and we thank you for this. We are pleased and honored to be here.

I doubt if there are many people in the United States who have not heard of Buffalo in one way or another. I noted recently in the Congressional Record that an industry destined for growth and expansion in Buffalo is the macaroni industry. All of us have eaten macaroni, but perhaps we didn't realize it may have come from Buffalo. This, I am sure, is but one of the many flourishing businesses found here. The availability of raw materials, utilities, and manpower tend to make this area a thriving community.

In my own case, the name of Buffalo makes me think of the romantic past of early America, the frontier, tales of the Erie Canal. It makes one think of marriage and of a honeymoon. To us out West, Buffalo is known from the television newsreels as a place sometimes blanketed by snow in winter. And for some of us, the strains of "Shuffle Off to Buffalo" evoke pleasant memories.

But it is strange that I have never met any people from Buffalo. As a matter of fact a young man in my community recently moved from the beautiful State of California to make Buffalo his home. The reason must be that Buffalo is a good place to stay and live one's life.

I understand that it has been 65 years since our organization has met in Buffalo. With 50 states in the Union now and with rotating our meetings among these states, it takes longer for us to come back to any one state again. But we're here and we're glad to be back.

All of us recognize that a very important part of our national heritage traces back to the people and the government of the great State of New York. History records that nearly one-third of the battles of the Revolution were fought on New York soil and in New York waters. From the earliest days of our nation's history, New York has pioneered and has led the way for a better agriculture and a better way of life for all Americans. You have given us the precedent for many things in our lives.

Since our Association has met here in New York, much has been done in improving the livestock health and milk and meat hygiene situation in our country. This is the primary interest of this Association. For instance, the great bovine tuberculosis eradication program was launched and the State of New York has done a tremendous job in reducing the
incidence of this disease under some very difficult circumstances. Other animal health programs have come along, and New York has been doing its part.

We are indebted to your New York State Veterinary College, Cornell University, for the excellent research and leadership provided to help overcome many of the animal disease problems with which we as an Association have been concerned. We also recognize the fine veterinarians who have graduated from this university and have come into our respective states to take their place and add their influence to the advancement of our society.

Many of us here today have visited New York State before while some of us have not. It may surprise you, but I am in the category of those members visiting your state for the first time.

Most of us, I presume, have preconceived ideas about places we have not visited. In many instances our conception is based on what other people have had to say or write or on pictures we have seen. But the great value of this visit is that we newcomers can see for ourselves what the "real" New York State is like.

And what I have seen thus far has impressed me greatly.

I have seen a few of your 66,000 farms. I understand better why New York stands 15th in the nation in gross farm income for a total of $939 million. I can see now why New York stands third nationally in the number of milk cows; 14th in chickens; 20th in all cattle and calves; 28th in turkeys; 30th in the number of stock sheep and lambs; and has significant numbers of other livestock.

Despite its immense population, its great number of factories and business enterprises, New York is still a powerful agricultural state.

It is fitting, then, that we hold this meeting in Buffalo, in New York. In doing so, the United States Livestock Sanitary Association salutes the Empire State.

Dean Poppensiek and Commissioner Wickham, again we thank you for your welcome to Buffalo. This will be, I am sure, a visit we will long remember.
PRESIDENT'S ADDRESS
C. L. Campbell
Tallahassee, Florida

"This Association was organized over 50 years ago by a group of men occupying positions very much the same as you and I occupy today. Then, as now, livestock producers of this country were faced with the problem of how to overcome economic losses occasioned by infectious and contagious diseases. Looking back, from our advantage of years and experience, we think their problems of cattle fever tick and scabies eradication were minor compared to the problems and losses confronting the livestock industry today. Since its organization in 1897, this Association has endeavored to correlate scientific information, sound thinking and practical experience in infectious livestock disease control and has become the agency which, through the years, has been largely responsible for formulating and conducting livestock sanitary disease control programs necessary to effect a profitable livestock industry and maintain a balanced agricultural economy.

"How long this Association remains the effective agency it has been in the past will be determined by the manner in which it meets and solves the problems confronting it from year to year."

These statements made at the 52nd Annual Meeting of this Association by the late Dr. J. V. Knapp, my predecessor at the time, are as applicable today as they were in 1948.

It is readily discernable through the increased list of standing committees appearing on your program this year, or at least which should appear on the program, that this Association has taken on added responsibilities. In addition to the newly created Committee on Salmonellosis, in view of the existence of the problem in many parts of the world today, I felt it imperative to reactivate the Committee on Vesicular Diseases. Moreover, the Committee on Regulatory Veterinary Resources has been established to implement certain recommendations of our past president, John Safford. The scope of activity of this appointed group, I felt, should be limited to surveying and gathering information upon which to base recommendations to the President and Executive Committee that involves the utilization of veterinary manpower and services. In his presidential address, Doctor Safford stressed the critical point which will be reached by 1980 due to the veterinary manpower shortage, unless maximum efficiency is obtained in the utilization of veterinarians. It is my feeling that because of the magnitude of this problem, a study of this scope must not be undertaken lightly. It must be so thoroughly and judiciously explored as to preclude any possible connotation of "witch hunting" in the fields of possible duplication as regards state and federal personnel. With this in mind, I urge my successor to continue during the next year the same balance of scope and area of responsibility in his appointments to this
Committee as are presently represented. The experience these members have received during this year of study should be invaluable in formulating definitive recommendations to the Executive Committee.

The final committee appointed this year is one of the most important created by this Association since its inception. It represents the consummation of a directive given by the Executive Committee to the officers of inquiring into the duties and affairs of this organization and its leaders, with recommendations for consideration at this year's Executive Committee meetings. While the original motion pertaining to this matter was directed to the proposition as to what should properly constitute the specific duties, responsibilities and remuneration of our Secretary-Treasurer, the officers concluded, following an exhaustive study of the matter, that an organization such as this, which has attained such respected world recognized scientific stature as ours, should have a complete "revamping" if this status is to be maintained. It was concluded that a comprehensive study of its structure, overall affairs of the organization, as well as its future should be carefully scrutinized by a select group of men whose association with the United States Livestock Sanitary Association through their having previously served as officers, provided them with the capability of efficiently coping with the problem. To this end, the "President's Interim Committee on Association Affairs" was named and will present an initial report to the Executive Committee at this afternoon's session.

As President, I wish to express my appreciation to and commend the gentlemen serving on this Committee in accepting and implementing this most difficult assignment, and I would urge the most conscientious study on the part of each member of the report of this committee, which includes numerous proposed amendments to our Constitution and By-Laws, one being a change in name to that of the "International Animal Health Association," a title which now more nearly delineates the scope of our organization.

In recognition of the increased stature of our Association in recent years and the added responsibilities of the organization in the formulation of national and oftentimes world wide disease control and eradication programs, I have for several years felt that the time which we are allotted at our annual meetings is insufficient to accomplish that which of necessity must be accomplished if we are to continue as leaders in our role of keeping the nation's livestock healthy. As a consequence, it is my recommendation that the Program Committee give this matter sufficient import in planning next year's meeting. I feel it imperative to allow ample time for consideration and discussion of possible numerous changes which may ensue at our next meeting affecting the Constitution, By-Laws and policy making procedures of this group.

During the past year livestock regulatory officials, practitioners, veterinary associations, and state racing commissioners responded to the clarion call of the equine industry in temporarily putting down a problem which for a number of decades has plagued horse owners and breeders. Thus was developed, largely as a result of the response by many of you, what is now known as the "Prospectus on Equine Infectious Anemia with
Guidelines." I know that this has received, and while at this meeting will receive further consideration and criticism in the development of practical methods of dealing with swamp fever of horses. Obviously, as with any disease of this nature, the answer to its eventual elimination rests in the disclosure of a practical diagnostic test with subsequent application of remedial therapy. Such answers have now successfully been developed on a disease which until six years ago was considered exotic to the United States, that being equine piroplasmosis. There is absolutely no reason why, with the knowledge we have of this disease at the present time, that similar determinations cannot be made on equine infectious anemia at an early date. Financial assistance has been made available by the Congress and industry for instituting "crash" research directed to this end and we, therefore, cannot fail to accept this challenge and respond to our full capabilities as scientists.

With accelerated air travel shortening the distance to hours between our world's eastern and western hemispheres, the United States is increasingly confronted each day by potential exotic disease outbreaks. This of course has been apparent to this organization for some time, and for at least six years to my knowledge, our Committee on State-Federal Relations has been imploring the United States Department of Agriculture and Congress to shore up our defenses in this area through the construction of vastly improved import quarantine facilities at the Miami port of entry, a Florida port which has increased tremendously in foreign animal import dimensions during this past decade. It is, therefore, noteworthy to mention that within the past month negotiations have been completed between the United States Department of Agriculture and the Dade County Port Authority for the construction of an approximate $1,000,000 tight security import-export station at the Miami International Airport, which is slated for completion around the end of next year.

A year ago state regulatory officials were embroiled in a controversial issue centered around the so-called "Compendium of Ideas" involving inspection of meat and meat food products. This compendium, dealing with consumer protection, was so designed as to abrogate the voice of state government almost entirely in its inspectional programs through extensive application of federal meat inspection. The proposed bills developed from this compendium purportedly are drawn for improving the federal meat inspection program, but in certain sections extend federal inspection far beyond its proper confines. All of you with any knowledge of the nation's meat industry are well aware that meat and meat food products are an important source of the nation's economy and total supply of food, and it is essential in the public interest that the health and welfare of the consumer be protected through assurance that meat and meat food products distributed to them are wholesome. This knowledge, though, is not an endorsement of the notion that the only means of accomplishing this is through the broad federalization of meat inspection. Federal jurisdiction should be limited to activities which in fact involve interstate or foreign commerce. If we accept this as our credo, we are then faced with the crux of this problem: to provide an acceptable
inspection service on state and local planes of such standards as to prevent interference from the federal level. If we as state regulatory officials fail to recognize the need to act, we are certain to see the federal government rush into this vacuum created by our failure. It now appears that the bills which have been prepared to extend federal authority in this field are tabled at least temporarily during this session of Congress. But they won't remain tabled if those states with deficient programs fail to make the necessary corrections. Certainly each state regulatory official has a right to resist unnecessary interference from federal government levels. None of us has a right to evade his own responsibilities.

This may well be the last Presidential Address to be heard at our Association meetings if we are to follow the recommendation of last year's president in allowing the president-elect an opportunity to present the objectives which he hopes to attain during his term of office, in lieu of hearing the accomplishments of a "lame duck" president. This being a year of transition, we have provided in today's program an opportunity for both of these officers to be heard. Therefore, as one of the final official acts of this year's President, I wish to express my appreciation to those appointees who so graciously agreed to accept the chairmanship of the various committees during my term of office.

For me this moment is the culmination of four years of hard but most gratifying work in behalf of this organization, and I am truly grateful for having been allowed an opportunity to represent you in this capacity. Naturally, I could not have accomplished what little I have during my tenure without the dedicated assistance of the other officers with whom I served, and I am most appreciative for their devotion to the Association. While I am relinquishing the gavel to one whom I consider most qualified to assume the role of President, I will continue to work unceasingly in behalf of the goals of our organization.
ADDRESS OF PRESIDENT-ELECT
G. S. Kaley
Albany, New York

Distinguished guests, ladies and gentlemen:

My appearance here this morning marks a departure from the custom of the past. It is the first time that a President-elect has been called upon to address you as such. The lack of precedent left me in something of a predicament. Since my final status in the administrative scheme will not be definitely determined for another forty-eight hours, I was uncertain as to whether my remarks should assume the character of an inaugural address or of a campaign speech.

I have Dr. J. W. Safford to thank for this situation. It is his suggestion of a year ago that is being followed today.

The 70 years during which this Association and its predecessor organizations have functioned have been marked by continuous progress in the war on the communicable animal plagues that beset or threaten our livestock economy. I do not need to list for this audience the diseases which were once here but are no longer here or those which once produced economic disaster on countless farms but are now controlled to the point where they are of comparatively minor significance. You are well aware too, that the battle lines have been drawn against still others.

Now some of this progress would of course have been made even though this organization had never existed. However it would have been on a much less imaginative and ambitious scale and, without full coordination, the goal would have been limited to regional control as opposed to area eradication. It is to this end the United States Livestock Sanitary Association had dedicated its efforts.

In view of this record of solid achievement, no prudent President-elect is going to recommend major surgery on a patient as basically healthy as this Association. There are, however, details of procedure which have evolved over the years which could well be the subject of critical analysis because of their effect on the growth and internal strength of the organization. Even here there is need for restraint because I have no desire to anticipate the report of the Interim Committee on Association Affairs which President Campbell mentioned a few minutes ago. This report, presumably, will concern itself with those same areas and same details I have in mind. I await the report with interest because it will represent the composite and considered opinion of a group whose knowledge of the Association and its problems is unique. Therein, I am sure, we will find the guidelines for the years immediately ahead.

This brings me to my first concrete suggestion, which is to propose that the Committee on Nominations and Resolutions become the Committee on Nominations, Resolutions and Internal Affairs. It is my feeling that
with this broader functional base, the experience of this group could con-
tinually be brought to bear on matters relating to the structure and well
being of the Association. The views of the Committee in this regard could
be presented in the form of informal observations, admonitions, sugges-
tions and recommendations on the course which Association affairs might
be taking. This guidance could be invaluable to the officers and to the Ex-
cutive Committee.

Another area of concern of which I do feel free to speak is member-
ship. It seems to be that the intrinsic strength of an organization such as
this lies not in the officers or Executive Committee but in an interested,
actively participating, general membership. It is best if this membership
be diverse and truly representative of all phases and segments of the
livestock economy. The danger of gradual and insidious over-orientation
toward some particular aspect of livestock disease control is very real
and is to be guarded against. In this general connection I commend to
your careful and studied scrutiny, Article II of the United States Livestock
Sanitary Association Constitution in which the purposes of the Association
are set forth. They number about a half dozen and each would appear to
have equal status. If special emphasis is placed on one or two of these
objectives, it should not be done at the expense of the others. Failure to
diligently pursue all objectives can only leave a vacuum into which some-
one else will soon move.

It might be well at this time to examine our existing procedures with
respect to general membership with a view to determining how well they
lend themselves to sustaining member interest and encouraging member
participation in Association affairs.

For the purpose let us take the hypothetical case of Joseph L. Doaks.
All his friends call him Joe so we will take the same liberty. Joe may be
a veterinarian, a livestock owner or a bacteriologist engaged in Salmonella
research. He might reside in Potter County, South Dakota, Frederick
County, Virginia or Mason County, Michigan.

At any rate Joe somehow hears about the United States Livestock
Sanitary Association and in January 1967 sends an application card to
Trenton, New Jersey along with a check for $5.00. Now the Constitution
and By-Laws make it clear that the application must be acted on by the
Executive Committee which, in 1967, will meet for the first time in Phoe-
nix, Arizona along about October 18.

So, in the normal course of events, Joe will get his membership card
sometime in November, which is approximately ten months after applica-
tion. In fact, he may get another application card and a bill for his 1968
dues before he knows how his original application stands. If in the
process, Joe’s interest in the whole matter has largely evaporated, who
can blame him?

I would suggest that the Constitution and By-Laws be modified to per-
mit acceptance of applications on the recommendation of a single member
in good standing without further consideration of the matter by the ex-
cutive committee.

Now let us suppose that this change is made. Thereafter the Joe
Doaks—I like to think there are, potentially, several thousand of them—can quickly and easily become members. Our particular hypothetical Joe will get his membership card next February. This is something gained. However, his next contact with the Association will not come until about Labor Day when he receives a program for an annual meeting perhaps a thousand miles from home. In the meantime the Association has exhibited no particular interest in Joe, his interests or his background. It has made no effort to keep him posted on what is going on. To Joe, at this point, that thousand miles can look like a very long way. Even if he does get there, it is unlikely that his talents will find an outlet in committee service because the Association has taken no steps to determine just what those talents might be.

It seems to be that if the individual is to take an interest in the Association, the Association must indicate an interest in the individual. This is an area that has been too long neglected. With a view to sustaining member interest I suggest that a newsletter of about four pages, and of appropriate format and content, be mailed to each member four times each year but preferably during the first week of March, June, September and December respectively. Among other things, the June and September letters could include or be accompanied by information on the forthcoming meeting and the meeting programs could be inserted in the September mailing without additional expense.

The letters could include items of interest from the states or gleaned from research journals or other publications. One of them could include a short biographical sketch of new member Joe Doaks. They could lend themselves in various ways to promotion of the Association image. They could be punched to facilitate permanent filing in a standard three ring binder and the pages could be serially numbered for ready reference.

I would propose that the officers of the Association be empowered to appoint an editor whose responsibility it would be to compile suitable material and to prepare the original drafts of such letters for submission to the Secretary-Treasurer by the 25th of the month proceeding publication. The Secretary-Treasurer would reproduce and mail.

The editor should be adequately compensated for his services at a rate to be determined by the Executive Committee. The services of the editor should be subject to termination at anytime by a majority vote of the Executive Committee. In such event, the officers would select and employ a successor. Rotation of editors on a three year basis to assure a constantly fresh and vigorous approach might be considered.

A moment ago I mentioned that biographical sketch of our friend Joe. I believe that such a record should be on file for every member, present and future, and that this record should be kept current. It would be of particular value in screening the membership for committee assignments. I am sure that we have overlooked a lot of talent in this organization that could be used to better advantage in the interest of the nation's livestock economy. Further, judging from my own experience, we sometimes get the square pegs in the round holes. For several years past it has been my privilege to be a member of the Committee on Animal Virus
Characterization. While it was an honor to be listed with that learned group, the fact is that by all the criteria of training, experience and aptitude, this was a role for which I was completely and eminently unqualified.

A broader as well as a more selective base from which to make committee appointments is also desirable. Some expansion of committees would permit active participation by more members. The committee reports themselves, hopefully, would not be expanded in proportion. It would also seem advisable to arbitrarily restrict appointments of any individual to one or at most two committees. No one can do full justice to the work of three or four committees. The spreading of too few people over too many committees can give rise to situations such as that described in one committee report last year. Only the Chairman was present. All the members were occupied elsewhere with other committee work at the time the meeting convened. With all the talent we have in this organization, there is no need for such things to happen.

Consideration might be given to appointing the Chairmen of standing committees on a staggered basis, for periods of three years. Each incoming president would then appoint about eight instead of two dozen chairmen. Any limitation which this might place on the President would be compensated for by continuity of committee approach to the subject at hand. I can assure you too, that as a practical matter, the President's present freedom to move in this area is much more limited than might appear to be the case.

It can be expected that the Chairmen themselves would function better with the degree of security afforded by a three year tenure. At the same time the incoming President would feel entirely free to openly contact his replacements well in advance of the annual meeting because the incumbent chairman would be completely aware that his period in the chair was approaching an end and that replacement was inevitable. Committees for the coming year could then be organized prior to the annual meeting, this organization could be firmed up with a few last minute changes at the annual meeting and committee lists could be reproduced and published before the session ends.

These thoughts and suggestions are offered in the interest of smoother internal operation, greater appeal to the prospective member, sustained member interest and a more active and productive participation by the average individual. I ask that each of you weigh carefully in your own mind the possible advantages and disadvantages of each.

There are other details of Association affairs which might well be discussed at this time, including the ever important subject of finances. However, I am sure that the Interim Committee report will touch on these matters and I feel that we should await those suggestions.
Mr. Chairman, Ladies and Gentlemen:

I am very pleased with the registration at this time which numbers over 375 and bids fair to set a new all time record for our Association.

I shall endeavor to make my report as brief as possible because, as you will note, the program calls for the presentation of a plaque to each of the fifteen living past presidents and it may take a little time.

Since January your Secretary attended many meetings. On January 17 the Meeting of the Western States Regulatory Officials in Las Vegas. This was a very interesting meeting held in conjunction with the Intermountain Veterinary Medical Association.

The major topics discussed were: First, the State Meat Inspection Program and its relation to the proposal of the Consumer and Marketing Division of the United States Department of Agriculture. Second, there was a lively discussion about the occurrence of scabies in certain states in the Western Group. Third, of particular interest to me was the discussion attendant the change in the name of Western States Regulatory Officials to that of Western States Animal Health Association. I have tried repeatedly over the past 24 years to get our Executive Committee to seriously consider a change in the name of our Association to one more meaningful than United States Livestock Sanitary Association. As a matter of fact there is a proposed amendment to be considered this year which asks the name be changed to American Animal Health Association. I feel confident that the inclusion of the magic word "Health" in our name will be of help in legislative halls as well as a great saving of time and verbage in explaining just what line of endeavor we are engaged in.

The Western States Group are to be congratulated in the change in name of their Group. State Departments would be smart to give consideration to a change in the name as has Texas which has renamed their Animal Industry Division the Texas Animal Health Commission.

On the way to the Las Vegas Meeting Doctor William Thompson, then State Veterinarian of Arizona, and I visited the Westward-Ho Hotel in Phoenix to check on arrangements of our 1967 Annual Meeting.

On February 7, 8, 9, I attended the Annual Meeting of the National Mastitis Council at the Sahara Motel at O'Hare Field, Chicago. This was an exceptionally well attended meeting, some 300 persons present. The Mastitis Programs of several states were presented and we heard discussions by dairymen and veterinary practitioners concerning the merits of the official plans. Listening to the presentations and discussions one had the feeling that, while infection and inflammation of the mammary gland is one part of the program, primarily this is a potable milk program in which the major factor is management.
Basicallly there is a lot of educational effort that needs to be made and the Colleges of Agriculture can do much in the way of demonstrating proper care and management of dairy animals and its value in producing a quality product. The prospective dairyman should be taught to examine the placement of teats in the calf, the prevention of suckling, the development of the udder during heiferhood, the physiology of the udder and the mechanics of milk letdown. In my experience too often the important act of preparing the udder for milking and the actual milking operation was relegated to uninformed or improperly informed and dedicated persons. Even the gentle handling of milk cows has an effect on udder health. One would be surprised what effect a radio in the barn can have on production in a milking herd.

At a meeting called by the Chairman the Committee on the Nationwide Eradication of Hog Cholera, of which I am a charter member having inaugurated the Committee in 1951, met and discussed with State Officials of Iowa, Illinois, Indiana and Missouri, adjustment of the disposal of apparently healthy swine originating in infected herds. It was debated that the rapid movement of exposed swine to slaughter for regular kill was a calculated risk that may pave the way to early economical eradication of hog cholera in the states involved.

On February 18 and 19 our President called a meeting of regulatory officials and representatives of the horse industry in Atlanta, Georgia, to review a proposal to assist the industry in the prevention of the spread of Equine Infectious Anemia. This meeting was well attended and served to inform industry the seriousness of the problem and our lack of a definitive test to identify infected animals and carrier cases.

February 21 and 22 was spent in attendance at the L. C. I. Meeting held in Kansas City. I attended the disease control meetings of this group and also a meeting of the Committee on Hog Cholera with the four Midwestern States at which time we revised the provisions of phase 3.

On March 14 and 15 the Officers of the Association, as instructed by the Executive Committee at the 69th Meeting, visited my residence in Trenton, New Jersey, and reviewed with me the method of conducting the business of the office of Secretary-Treasurer.

March 16 and 17 was spent in Washington, D. C. attending a meeting of the Committee on State-Federal Relations and our appearance before the Officials in the United States Department of Agriculture at which time we presented our view of the work being performed in respect to cooperative disease control and eradication programs, the testing and control and checking of biologic products and the adequacy of our system of prevention of the introduction of foreign animal disease and/or their causative agents.

April 4, 5 and 6 was spent in attendance at the Meeting of the North Central Veterinary Regulatory Group at the Sherman House in Chicago. This meeting was well organized and conducted with an agenda which covered most of the conditions prevalent in animals in that area.

April 17 was spent in attendance at the Southern States Regulatory Group Meeting in Atlanta, Georgia.
June 26 to 29 was devoted to attending the 38th Annual Meeting of the Conference of the Northeast Avian Diseases, formerly and originally the Northeastern Pullorum Conference, at Dover, Delaware. There were many valuable papers and discussions presented concerning infectious diseases of birds.

July 10 to 14 was spent attending the American Veterinary Medical Association Meeting at Louisville, Kentucky, where I made arrangements for the Association's annual breakfast for the Committee on State-Federal Relations. At this breakfast meeting members of the Agricultural Research Service of the United States Department of Agriculture were present and they reviewed with our Committee on State-Federal Relations just what was accomplished in the way of support for cooperative disease control and eradication programs and animal disease and parasite research.

On July 28 I spent the day in conference with Messrs. J. McMahon, Mike Murray, Robert Guise and others at the Statler-Hilton Hotel in Buffalo, New York, in assigning committee meeting rooms, in fact all rooms and necessary appurtenances needed to provide a smooth running of the 70th Annual Meeting of the Association.

As you can see this year has been one in which of necessity I have been away from my office considerable time.

It has been my pleasure to serve you as Secretary-Treasurer, and I wish at this time to thank the officers and members of the various committees for their cooperation during the year and currently for the development of the excellent program both educational and entertainment which we are privileged to enjoy. I wish also to pay my compliments to the conference of Veterinary Laboratory Diagnosticians for their excellent program and to the Statler-Hilton Hotel personnel for their cooperation. Should anyone have need of assistance from the Hotel I am confident Mr. Counts and his staff will be found very cooperative. My thanks to Dr. H. E. Nalder and members of Dr. G. S. Kaley's staff for help in public relations and many other ways to ease my responsibilities in connection with this meeting. A special thank you to the Haas Family who planned the usual excellent social hour and also to Doctor Powers and his son and the typists for assistance on the registration desk.

Following is the Treasurer's Report as prepared by C. B. Groendyck, C.P.A. Trenton, New Jersey. I invite your questions concerning each item in the report. I am prepared to explain and justify every item.
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR THE PERIOD FROM OCTOBER 1, 1965 TO SEPTEMBER 15, 1966

CASH BALANCE, October 1, 1965:

First Trenton National Bank, Trenton, N.J. $ 22.31
Trevoise Savings and Loan Association, Morrisville, Pa. 1.00
Sandia Savings and Loan Association, Albuquerque, New Mexico 4,364.96

Total Cash Balance 4,388.27

INCREASED BY CASH RECEIPTS:

Individual Dues 6,280.91
Official Dues 5,409.00
Proceedings 2,527.38
Reprints 2,518.62
Registration Fees 4,080.00
Foreign Animal Disease Handbooks 4,258.19
Interest on U. S. Treasury Bonds 800.00
Interest on Sandia Savings and Loan Account 183.22
Interest on Trevoise Savings and Loan Account 125.57

Total Cash Receipts 26,473.89

DECREASED BY CASH EXPENDITURES:

Meeting Expenses 922.31
Printing and Stationery 8,185.86
Salary 7,500.00
Communications 704.81
Travel 2,165.62
Electricity 106.02
Rent 420.00
Insurance 159.57
Miscellaneous 7.83

Total Cash Expenditures 20,818.55

CASH BALANCE, September 15, 1966:

First Trenton National Bank, Trenton, N.J. 63.18
Trevoise Savings and Loan Association, Morrisville, Pa. 5,432.25
Sandia Savings and Loan Association, Albuquerque, New Mexico 4,548.18

Total Cash Balance $10,043.61
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS
FOR THE PERIOD FROM OCTOBER 1, 1965 TO SEPTEMBER 15, 1966

REVENUE:
Total Cash Receipts $26,473.89

Decrease in Accounts Receivable:
Accounts Receivable - Sept. 30, 1965 $1,454.00
Accounts Receivable - Sept. 15, 1966 1,308.24

145.76

Total Revenue (Accrual Basis) $26,328.13

EXPENDITURES

20,818.55

NET REVENUE FROM OPERATIONS FOR FISCAL PERIOD $ 5,509.58

NET WORTH - SEPTEMBER 15, 1966

Accounts Receivable $ 1,308.24
Balance, First Trenton National Bank, Trenton, N.J. 63.18
Balance, Trevose Savings and Loan Assoc., Morrisville, Pa. 5,432.25
Balance, Sandia Savings and Loan Assoc., Albuquerque, N. M. 4,548.18
U. S. Treasury Bonds, 4%, Due February 15, 1980 20,000.00
Furniture and Fixtures 400.00

NET WORTH, September 15, 1966 $31,751.85

ANALYSIS OF CHANGE IN NET WORTH

Net Worth, October 1, 1965 $26,242.27

Increased by:
Net Revenue from Operations for Fiscal Period ended September 15, 1966 5,509.58

Net Worth, September 15, 1966 $31,751.85

I would move that the Report of the Secretary-Treasurer be referred to the Executive Committee. Thank you.
THE PRESENTATION OF AWARD PLAQUES TO LIVING PAST PRESIDENTS

Dr. R. A. Hendershott
Trenton, New Jersey

Mr. Chairman, Ladies and Gentlemen:

In 1950 I had a key designed for our Association, a replica of it is imprinted on the front cover of the paper-bound proceedings and upon all reprints. Since the Fifty-fourth Annual Meeting gold keys have been presented to all living past presidents, and annually to the retiring president. A year ago the thought occurred to me that we should also present to the outgoing president a plaque properly inscribed and signed by the incoming president and the secretary-treasurer of our Association.

Last year Doctor John Safford was the first recipient of such a plaque, mounted on wood and plasticized. It was done in ink of attractive colors and was admired by those who saw it.

It was my intention to provide each living past president with a similar plaque. Today we have one for each of the living past presidents, many of whom are present. Most of these men are well known to a great many of you in attendance at this meeting. They served our Association with distinction and each in his turn did his part to advance the prevention, control and eradication of diseases of livestock and poultry and is deserving of our Association's acknowledgement in a manner that is set forth in the wording of the award which reads as follows:

The United States Livestock Sanitary Association presents this Certificate of Appreciation to _______ President, in acknowledgement of the services rendered to this Association, to the livestock and poultry industries, to the health of animals and to the health and welfare of man, during his term of office. As an expression of the esteem in which Dr. or Mr. _______ is held by the members of this Association he is hereby commended for his distinguished service as President.

Signed by President Elect _______ and _______ Secretary Treasurer

I consider it an honor and privilege to have served with these gentlemen and more particularly to have the opportunity to personally present these awards. When I call the name of a past president in event he is not here will the State Veterinarian of his State accept the plaque and convey it to him. As I call out the names will the men please come forward and receive their plaques. Since time is short I trust we can dispense with acceptance speeches. Thank you.

Dr. W. L. Hendricks, Utah 1943
Dr. J. M. Sutton, Georgia 1944
Dr. C. U. Duckworth, California 1945
Dr. C. P. Bishop, Pennsylvania 1950
Dr. R. L. West, Minnesota 1952
Dr. H. F. Wilkins, California 1955
Dr. A. L. Brueckner, Maryland 1956
Dr. G. H. Good, Wyoming 1957
Dr. J. G. Milligan, Alabama 1958
Mr. F. G. Buzzell, Maine 1959
Dr. J. R. Hay, Illinois 1960
Dr. A. P. Schneider, Idaho 1961
Dr. W. L. Bendix, Virginia 1962
Dr. T. J. Grennan, Jr., Rhode Island 1963
Dr. L. A. Rosner, Missouri 1964
Dr. C. L. Campbell, Florida 1966
REPORT OF THE COMMITTEE ON LAWS AND REGULATIONS

G. B. Rea, Salem Oregon, Chairman; D. E. Flagg, Bismarck, North Dakota; F. W. Hanson, Jr., Hyattsville, Maryland; J. F. Huddelson, Topeka, Kansas; T. A. Ladson, Olney, Maryland; D. L. Smith, Indianapolis, Indiana; W. M. Thompson, Phoenix, Arizona

The usual semi-annual meeting held in conjunction with the American Veterinary Medical Association did not materialize this past year, due primarily to the transportation difficulty brought about by the airline strike. Several subjects have been discussed by correspondence and in Committee meetings and are presented herewith for your consideration.

Identification of Livestock and Interstate Commerce.

We have reviewed the report of 1965 and find that only one item referred to therein continues to require our active participation. This is the subject of "Identification of Livestock in Interstate Commerce." In July, 1966, Dr. D. A. McGill, Chairman of the Committee on Stockyards, Market and Transportation, joined us in appointing a joint subcommittee to study proposals and to make suggestions for improving our surveillance of the movement of livestock in interstate trade. The present back tagging system gives us an approach to part of the problem and is presently being used as a tool in the Brucellosis and Tuberculosis programs. Although proving of great assistance in promulgating the eradication of these two diseases, considerable improvement must be made before back tagging will be completely effective. With the back tags we are speaking only of the interstate identification of eligible cattle and have not addressed ourselves to the identification of any other species in interstate movements. Since this subject is more directly related to the responsibilities of Stockyards, Markets and Transportation, we refer you to that Committee's report for the results of the joint subcommittee studies.

Equine Infectious Anemia.

This subject was discussed but since it is being considered by the Committee as Infectious Diseases of Horses we have determined that recommendations should come from that group.

Interstate Livestock Health Regulations and Livestock Movement.

Last year's Committee strongly urged the ANH to use the recently adopted uniform Health Certificate in lieu of the ADE 2-48 and other forms for the release of Livestock from federal yards.

We are pleased to report that such a form has been devised and is being issued at this time for this purpose. We applaud the ANH for this action.

It is noted that approximately 20 states are presently using the United States Livestock Sanitary Association approved Health Certificate form.
We continue to urge the adoption of this Certificate by all other states as rapidly as possible.

*Forwarding Interstate Copies of Health Certificates.*

President Campbell directed our Committee to study a suggestion made by one of the states, concerning the copy of the health certificate which is ordinarily sent to the state of destination by the official of state of origin. It was suggested that the issuing veterinarian send this copy directly to the official of state of destination rather than moving through the state veterinarian or state official of the state of origin. The thought behind this suggestion was that the direct forwarding of the health certificate from the veterinarian making the inspection would reduce the time lag which necessarily accompanies such paper work. The official making the suggestion stated that most of the time the cattle had arrived in his state and were dispersed before he had knowledge of the fact that a health certificate was ever written. Much discussion of this proposal was entered into by members of the National Assembly as well as by our committee members. Although the initial reaction was adverse, subsequent discussion brought out some merit in the proposal to the extent that this Committee on Laws and Regulations has undertaken the responsibility of considering a system of Health Certificate distribution that would decrease the "time lag" between the time of inspection and the time of receipt by the official of the state of destination and still maintain control over the Veterinarian issuing the certificate. The committee will study all proposals and suggestions presented in an attempt to find a solution to this problem.

*Standard Format for A.R.S. 91-17. "Health requirements governing interstate and international Movement of Livestock and Poultry."

The Committee on Laws and Regulations was asked to study a standard format to be used in future printings of the A.R.S. 91-17. This document as you will recall, is a compilation of all interstate regulations, and is published periodically by the ANH. The various laws and regulations of individual states would not be altered in any way but they would appear in this publication in a similar form for each state. Each state would be asked to interpret their own laws and regulations and fit them to this format for publication. Briefly stated there would first be a General Statement including:

1. Relation between these regulations and the federal requirements, if any.
2. Define who may inspect livestock for interstate movement.
   a. When permits are needed
   b. Where permits may be obtained.
3. State requirements concerning health certificates and/or permits.

There would then follow a section on each species broken down to show requirements for Specific disease and other movements. A copy of the
suggested format is herewith attached. The Committee on Laws and Regulations recommends the adoption of this suggestion by the several states as an aid in the use of the A.R.S. 91-17.

This report is submitted for your approval.
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

M. J. Twiehaus, Lincoln, Nebraska, Chairman; E. H. Bohl, Columbus, Ohio; L. E. Hanson, Urbana, Illinois; J. F. Huddleson, Topeka, Kansas; R. L. Morter, Lafayette, Indiana; C. S. Roberts, Auburn, Alabama; E. E. Roth, Baton Rouge, Louisiana; O. H. Stalheim, Ames, Iowa; H. B. Stoenner, Hamilton, Montana

Your Committee considered previous reports submitted by this Committee and recent data available on leptospirosis. The discussion during the two sessions was centered on the significance of serotypes other than *Leptospira pomona* in domestic animals, wild life and rodents.

The Committee again unanimously concluded that this disease is not amenable to eradication because of the various serotypes encountered, the wide range of hosts and the inability to detect the carrier state. The following serotypes, *L. pomona*, *L. hardjo*, *L. grippotyphosa* have been isolated from cattle and *L. pomona*, and *L. grippotyphosa* from swine in the United States. Isolations of *L. grippotyphosa*, *L. hardjo* and *L. canicola* have been made only recently in a few states. At the present time there are only a few laboratories conducting epizootiologic studies of leptospirosis. Serologic and clinical evidence suggest that infection of cattle and swine with serotypes other than *L. pomona* are not limited to one geographical area in the United States. Abortions appear to be one of the principle signs associated with infection of these serotypes and these may not be recognized in nonpregnant cattle or swine. However, the Committee considers infection with *L. grippotyphosa* and *L. hardjo* to be of economic importance in some of the larger herds on the basis of recent available information. The Committee would like to point out that adequate financial support has not been available for expanding or initiating epizootiologic studies of these two and other serotypes. This is a major limiting factor on the information needed to make sound recommendations.

The Committee therefore proposes that the Executive Board of the United States Livestock Sanitary Association request adequate money from Congress to be made available for investigation and research on this insidious disease.

Further data on these serotypes should be collected from several geographical locations in the United States. Accumulation of this data could possibly be best done by the various state experiment stations and diagnostic laboratories.

The Committee recommends that when diagnostic laboratories examine cattle and swine serum that several antigenic serotypes be employed. All laboratories are encouraged to use a maximum number of serotypes that are consistent with the capability of the laboratory. Serotypes that should logically be included are those that have been isolated in the United States and that were associated with clinical manifestations of disease—*L. pomona*, *L. grippotyphosa*, *L. canicola*, *L. hardjo* and *L.
It is recognized that serological reactions to autumalis occur in the sera of several species due to the antigenic interaction with other serotypes. Isolations have not been made to substantiate a relationship of *L. autumalis* with clinical leptospirosis.

With accumulation of newer knowledge from bacteriological, serological and clinical evidence, your committee recommends that temporary licenses be issued to interested biological companies to produce monovalent bacterins for immunizations of cattle against *L. grippotyphosa* and *L. hardjo* and swine against *L. grippotyphosa*. This Committee recommends that these bacterins be used only when clinical signs are manifested and supported by serological or bacteriological confirmation. It is further suggested that a continuing prophylactic program be initiated in the problem herds to provide protection against cyclic reoccurrence of the infection. Potency and efficacy testing of these serotypes was considered and the Committee recommends that serological evidence of biological activity be demonstrated in the species in which the bacterin is used.

The Committee is pleased that studies are underway regarding testing and efficacy of various biological products by a control agency and will be looking forward to a report on the study.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this Committee be continued.
REPORT OF THE COMMITTEE ON NOMINATIONS

J. W. Safford, Helena, Montana, Chairman; W. L. Bendix, Richmond, Virginia; F. G. Buzzell, Augusta, Maine; T. J. Grenan, Jr., Providence, Rhode Island; J. R. Hay, Western Springs, Illinois; J. G. Milligan, Montgomery, Alabama; L. A. Rosner, Shumate, Missouri; A. P. Schneider, Boise, Idaho; K. F. Wells, Ottawa, Ontario Canada

DR. JOHN W. SAFFORD: Gentlemen, your Committee on Nominations congratulate the Association in the election of Dr. Grant S. Kaley, President-elect last year and endorse him for president.

For President-elect we nominate Dr. John F. Quinn of Michigan.

For First-Vice-President, Dr. John L. O'Harra of Nevada.

For Second-Vice-President, Dr. Frank B. Wheeler of Louisiana.

For Regional Farm Representatives representing the Western States:

Mr. O. H. Timm of Dixon, California and Mr. A. O. Wilson of Hysham, Montana

Representing the Southern States:

Mr. J. Finley of Encinal, Texas and Mr. J. Nance of Alamo, Tennessee

Representing the North Central States:

Mr. J. R. Bishop of Indiana and Mr. Ward Van Horn of South Dakota

Representing the North East Region:

Mr. E. S. Bryant of Union, Maine and Mr. W. L. Henning of University Park, Pennsylvania

Mr. Chairman, I move the acceptance of this Report.

DR. C. L. CAMPBELL: Gentlemen, are there any nominations from the floor?

DR. REA: I move the nominations be closed and that the secretary cast the ballot for election of the men named.

DR. C. L. CAMPBELL: Is there a second?

Dr. W. L. Bendix and several others seconded the motion.

DR. CAMPBELL: All in favor indicate by aye, the usual sign. Opposed like sign. Motion carried.

DR. CAMPBELL: Will Mr. Buzzell and Mr. Powell escort Mr. Timm to the podium. Mr. Timm, it is a pleasure to welcome you back to another term as one of the industry representatives to this Association. Would you like to say a few words?

MR. TIMM: I do not think any remarks are necessary. I appreciate my election as a representative for the ensuing year.

30
DR. CAMPBELL: Is Mr. Bishop still with us? No? I guess he has returned to Indiana. Are there any other delegates present? No? I would ask Doctor Bendix and Doctor Grennan to escort Doctor Wheeler to the podium. Dr. Grennan recommended that the Second-Vice-President get rid of the beret he wore on the Niagara Falls trip and I would amend to provide that the beret inure to the outgoing president. (laughter)

DR. F. B. WHEELER: I noticed in the transfer of this neck mike several times that it appeared all acted like the French—they put their arms around each other. Last night I was disturbed about losing my hat (beret). In the middle of the night someone entered my room and tried to kiss me. I thought it was a maid who came to tuck me in. I didn't realize until she left that the hat was going out with her. I am surprised—I thought it was Chadwick; he is from the deep south too. I think I lost a little hair—I thought he made a doll. I thought he was mad because the Southern Delegates had finally gotten around to electing a fellow. I have been wanting to come up here all the time. You had Clarence and Bendix and John Milligan and naw me and I have been waiting to come up here all the time. I do appreciate the honor and I certainly hope I don't disgrace the good people of the South and I shall do my best.

DR. CAMPBELL: Thank you very much Frank. That is probably the longest acceptance speech of anyone in this organization.

It is now my pleasure to ask Doctors Rea and McGill to escort First-Vice-President John O'Harra to the podium.

Doctor O'Harra, it is an honor to welcome you as First-Vice-President. Congratulations.

DR. J. L. O'HARRA: Thank you Mr. President. It is certainly a pleasure to be here. The officers did an outstanding job this past year and I expect to support the officers during the coming year. Thank you.

DR. CAMPBELL: It is now my privilege to request Doctors Henshaw and David Smith to escort Dr. John Quinn, our President-elect to the podium.

Doctor Quinn, it is a pleasure to welcome you to this podium as President-elect.

DOCTOR QUINN: Thank you Clarence. I just want to say that I humbly thank you for this honor and I shall do my very best to live up to it.

DOCTOR CAMPBELL: I should like to call on Doctors Brower and Shook to escort our new President Doctor Grant Kaley to the podium.

Grant, this will be my last opportunity to make my voice known and I do wish at this time to express in my swan song my particular appreciation to those officers who preceded and who follow me for the assistance they have given me while I have served as President. I enjoyed serving with you and I would be remiss if I did not take this opportunity to extend my personal thanks also to a man who has been of great assistance to our State Federal Relations Committee and to our president's Interim Committee in the formulation and typing of many of these reports, namely my
assistant, Mr. Powell. I express my appreciation to him as I am sure many others have.

Gentlemen, with these final words on my part it is with a great deal of pleasure I turn over the gavel to you, Doctor Kaley. Good luck.

DR. KALEY: You know, really it would have only taken one of those fellows to get me up here. One of the toughest jobs I am going to have, and I am well aware of it, is to follow in the footsteps of the man who handed me this gavel. I certainly want to thank you all for this expression of confidence and I can tell you that it is particularly gratifying to me in view of the unfortunate rumors that were circulating last evening. The coming twelve months must be looked upon as a period of transition as regards the affairs of this Association. It appears at this time that Doctor Hendershott and I may be respectively the last Secretary-Treasurer and the last President of the United States Livestock Sanitary Association. This is not as ominous as it might sound because I am sure we will emerge at the end of this period as an organization with perhaps a new name but still dedicated to the same principles as in the United States Livestock Sanitary Association, respected and honored among those concerned with the physical welfare of our animal population.

I am sure I can count upon the solid support of the splendid group of officers. I am particularly pleased to know that Ralph Hendershott will be at my side with the wise counsel and guidance that derives from a quarter of a century of loyal service to the United States Livestock Sanitary Association.

Thank you very much.

Is there any old or new business to come before this assembly? If not the motion for adjournment is in order.

VOICE: I move we adjourn.

DR. KALEY: Second? All in favor say aye. We stand adjourned.
REPORT OF THE COMMITTEE ON PUBLIC RELATIONS

H. E. Nadler, Albany, New York, Chairman; E. L. Brower, Trenton, New Jersey; R. L. Knudson, Hyattsville, Maryland

If I were selecting a committee chairman, my first consideration for qualification would be experience. Doctor Campbell obviously used some other reasoning since I would have to be classed as a strict amateur in the publicity field.

Fortunately my colleagues on the committee filled some of the void and where we lacked experience we tried to fill with enthusiasm. I mention this only so you can lay our mistakes and omissions to inexperience or enthusiasm.

The place and dates of the annual United States Livestock Sanitary Association meetings are set three years ahead. This gives your publicity committee ample opportunity to meet months in advance, to outline that year's publicity agenda and begin their campaign early, hoping to attract enough attention so that members and prospective members will select that year's meeting as a must.

Samples of pamphlets and brochures describing the various attractions in New York State and especially the Buffalo area were obtained and at our first meeting those seeming most appropriate were selected for distribution. The selection turned out to be much easier than the obtaining, in the quantities we wanted. We ended up, however, securing about 6,000 pieces of literature extolling the virtues of New York State, Buffalo and Niagara Falls. So that no one would miss the objective of these colorful brochures we made one slight addition—every one carried the message: "United States Livestock Sanitary Association meeting October 9-14, Buffalo, New York." The two rubber stamps containing this little message were obtained early in our campaign and with the possible exception of the "fragile" stamp the post office uses to begin the destruction of packages, none ever received the work-out these got. We estimate this message went out on nearly 10,000 pieces of mail and literature. They are still in good working order and with slight alteration could go back into use if you will bring your meeting back to Buffalo in the near future.

Unless a large budget is made available for publicity, the State Veterinarian is the actual key to effective publicity for your organization. The United States Livestock Sanitary Association is too important an organization to keep secret and each State Veterinarian should use every opportunity to publicize through their periodic mailings not only the annual meeting but the achievements attained by your deliberations at these meetings. He should also make a concerted effort to increase the membership in his state.

Our first mailings were to each State Veterinarian and the chief veterinarian in Canada, Puerto Rico and the Virgin Islands. Included was a
sample letter describing the United States Livestock Sanitary Association, the meeting dates, a form for membership and requesting that they assist our efforts by making this a part of their future routine mailings to veterinarians and livestock owners. This, or an improved form, appeared in the next issue of the official bulletins from many states and your cooperation was sincerely appreciated.

The recent gimmicks in successful promotion have been tigers and sex. We never seriously considered tigers and out of deference to the dignity of the organization—although we realized it would handicap us—refrained from capitalizing on the latter. However, along in September we realized we had made a mistake. We should have used the sex pitch. We had failed to get the undivided attention of some State Veterinarians—but now it was too late. Routine mailings of State Newsletters were beginning to come through with 1/2 - 3/4 of a page blank. What a splendid opportunity to incorporate in this blank space the message of the United States Livestock Sanitary Association meeting that was then only a short time away. A few minutes would have added this to the mimeograph stencil and could have attracted a new member or an additional registrant at this meeting.

When this is multiplied by 53 it adds up quickly. We can't express too strongly our belief that each State Veterinarian must play an active and continuing role in bringing the United States Livestock Sanitary Association to the attention of interested people in the state.

Our initial efforts were directed toward getting members and prospective members to set aside the meeting dates in their busy schedule—maybe work the meeting in as part of a fall vacation. In short, get people to Buffalo.

Out next consideration—in case they showed up—what can we do for them after they get here. We knew a lot of people wanted to visit Niagara Falls and would take off sometime during the meeting in search of it. Maybe miss something they shouldn't.

Working under the premise "All work an no play is no damn good" and with President Campbell's counsel and guidance the tour and dinner at Niagara Falls were made a part of the program to give all an opportunity to visit the Falls and enjoy a short recess from your deliberations.

We realize every state does not have one of the seven wonders of the world at the door step of the annual meeting place. However, each state has places and natural wonders unique to that area that those attending would like to see and an arranged trip could make a refreshing addition and break in the program. They also have products, both agricultural and industrial, those in attendance would be happy to sample.

Being a committee of young men, it was inevitable that sooner or later our thoughts would turn to girls. Why not have a program for the ladies? Some might even bring along a reluctant husband to attend the meeting, again proving to him that her decisions always benefit his welfare.

There were other things we would have liked to do and will make a couple of them a part of the record for consideration by future committees.
One—a brochure should be prepared describing the aims, purposes and accomplishments of the United States Livestock Sanitary Association for distribution to members and prospective members.

Second—a concerted effort made to obtain more members from our good friends and neighbors to the north in Canada.

To carry out our publicity plans, we had wonderful cooperation and assistance from several individuals and Divisions in the New York State Department of Agriculture and Markets. Dr. G. S. Kaley and his office staff rendered yeoman's service. Without this help our program would have been severely curtailed and we extend a grateful thank you. We also extend our gratitude to Drs. Campbell and Hendershott and the State Veterinarians for their counsel and efforts in assisting us to publicize this meeting.

Working on this committee has been a pleasant sojourn into a field I knew nothing about and supplied an enjoyable diversion from the job one is supposed to know something about.

If our efforts have in any way contributed to the success of this meeting or added to the membership, we feel we have accomplished our goal and have been adequately rewarded.
REPORT OF THE COMMITTEE ON RABIES


As Chairman of the Rabies Committee, I wish to present this report on the incidence of rabies in the United States covering the calendar year 1965. At our last meeting in Lansing, the statistics revealed an alarming increase of 851 cases in 1964 over 1963. The picture is not quite as dark in the 1965 report, as statistics show a decline of 200 reported cases, and a 4% decline in the number of cases attributed to wildlife. However, the expected increase in rabies in our domestic animals as a result of the high incidence of the disease in wildlife is beginning to become apparent. This report shows 536 cases of rabid cattle for 1965. There appears to be a direct correlation of the infection between fox, skunk, and cattle. Iowa reported 118 cases of skunk rabies and 56 cases of cattle rabies; Texas had 165 rabid skunk and 39 rabid cattle; Illinois had 158 rabid skunk and 24 rabid cattle; New York had 100 rabid fox and 56 rabid cattle; Ten-nesssee had 394 rabid fox and 98 rabid cattle; Virginia had 239 rabid fox and 58 rabid cattle.

It is quite apparent that the problem is recognized. Our job now is to find the right answers through research, trial and error, and hard work with concerted effort.

In 1965 there was a total of 4,584 laboratory confirmed cases in the United States. This represents 200 fewer cases than in 1964, but a 20% increase over the previous five year average. Seventy-one percent of the total cases appeared in wildlife and again skunk and foxes were the most frequent infected species. Bat rabies accounted for over 10% of the total animal rabies cases. Kentucky, Tennessee, Missouri, and Illinois was an area of dog rabies along with the four states bordering on Mexico. For the first time in 28 years, all the New England states reported at least one case of rabies. Unfortunately, we must include one human death in 1965. A sixty year old man from West Virginia died 23 months after being bitten by a rabid dog. As to the epidemiology of rabies, no definite conclusions can be drawn as to whether there is real increase in rabies in wildlife or whether rabies is in a wildlife cyclic phenomon. However, at least a part of the increase seems to be due to a build up of the disease in wildlife species, as reported cases of skunk rabies increased from 319 in 1953 to 1,582 in 1965.

Research attempts to control the density patterns of wildlife are being made by various groups. Reproductive inhibitors have been screened and diethyl stilbesterol appears to be the most feasible drug. Conclusions have been reached that as the density pattern increases, the incidence of
rabies increases also. Therefore, reduction of a class of wildlife's ability to reproduce, warrants intensive study. The greatest limitation of a program of this nature is that treated baits must be consumed immediately prior to, or during the breeding season.

Trapping is economically feasible only where an immediate reduction in an already dense population is necessary. Poison bait has proven very desirable where a brush fire type of outbreak has occurred. Where an educational program beamed at the general public has been carried out, no objections were encountered by pet owners who were notified to confine their animals during the poison bait program.

Workers at the Communicable Disease Center in Atlanta have developed a self-triggering syringe which when placed in the travel routes of fox will inject a rabies vaccine into the animal.

Both state and federal conservation agencies, along with the public health departments, are quite concerned with the problem of rabies in wildlife because of the potential danger to the civil population seeking outdoor recreation. Some state agencies are warning campers, fishermen and hunters of the existing danger. The State of Wisconsin is posting recreation areas with signs warning the outdoor populace to watch out for rabid animals. Other states are restricting the movement of dogs in camp sites.

To bring this assembly up to date from the report of last year, there has been a rather sharp decline in the racoon outbreak reported from the various states. A drop to 99 cases from the previously reported 173 cases is encouraging. Seventy-seven (77) of these cases were reported from Georgia and Florida and again this year, as mentioned last year, no new cases were reported north of the Altamaha River.

Rabies in bats is still on the increase, climbing to a new high of 484 reported cases, a 27% increase over last year's 352 cases. The danger involved in this species is that small children in the suburbs think they are injured birds and pick them up. Two such incidences occurred in the Atlanta area in August of this year and both children received the rabies shots.

The greatest incidence of fox rabies occurred in Tennessee where 37% of the total 1,038 cases in the United States were reported. Last year the total was 1,061. No attempt will be made to explain the small difference in these two years.

The Committee is gratified to report a 17% decline in reported cases of skunk rabies. In 1964, there were 1,909 cases as against 1,552 for 1965. Iowa, Minnesota, North Dakota, South Dakota, and California accounted for a drop of 610 cases. However, increases in other areas partially off-set the over-all improvement.

The only significant change in the dog and cat picture was an increase of feline rabies from 220 cases in 1964 to 289 cases in 1965. Eighty (80) cases of this figure were reported from Illinois. It is gratifying to note that the Veterinary and Public Health Profession are keeping dog rabies in check. It would appear that with even more concerted effort in the way of community action and rabies clinics this figure would be depressed even further in the immediate future.
Raccoon Rabies U.S.

*69th USLSA Proceedings - 1965
Current Information - CDC

Graph I
RABIES

BAT RABIES U.S.

*69th USLSA Proceedings - 1965
Current Information - CDC

Graph II
In view of the fact that rabies is being reported in states where it has not been reported in many years, and that rabies in wildlife is increasingly becoming a national problem, the Rabies Committee will propose a resolution to the Resolution Committee which will embody the following recommendation: That the United States Livestock Sanitary Association immediately and vigorously promote the formation of a National Rabies Council which will bring under one group representatives from all other groups which are engaged in rabies investigation. It shall be the function of the National Rabies Council to gather together all information pertinent
TABLE I
Incidence of Rabies in U.S. by Type of Animal

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Cats</th>
<th>Farm Animals</th>
<th>Fox</th>
<th>Bats</th>
<th>Skunk</th>
<th>Other Animals</th>
<th>Man</th>
<th>Total</th>
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<td>5,688</td>
<td>538</td>
<td>1,118</td>
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<td>8</td>
<td>319</td>
<td>119</td>
<td>14</td>
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<td>462</td>
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<td>1,028</td>
<td>4</td>
<td>547</td>
<td>118</td>
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<td>924</td>
<td>1,223</td>
<td>14</td>
<td>580</td>
<td>98</td>
<td>5</td>
<td>5,844</td>
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<td>794</td>
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<td>484</td>
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<td>1</td>
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</tr>
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</table>

NATIONAL PATTERN OF RABIES AREAS

to the rabies problem. This Council will bring together those individuals who are knowledgeable in rabies control, research and epidemiology to make recommendations for the uniform control and ultimate eradication of rabies. This Council may wish to consider proposed legislation at the National, State, and local levels.
SKUNK RABIES U.S.

*69th USLSA Proceedings - 1965
Current Information - CDC

Graph IV
*69th USLSA Proceedings - 1965
Current Information - CDC

Graph V
REPORT OF THE COMMITTEE ON RESOLUTIONS

J. W. Safford, Helena, Montana, Chairman; W. L. Bendix, Richmond, Virginia; F. G. Buzzell, Augusta, Maine; T. J. Grennan, Jr., Providence, Rhode Island; J. R. Hay, Western Springs, Illinois; J. G. Milligan, Montgomery, Alabama; L. A. Rosner, Shumate, Missouri; A. P. Schneider, Boise, Idaho; K. F. Wells, Ottawa, Ontario, Canada

RESOLUTION FOR STATE-FEDERAL RELATIONS COMMITTEE

WHEREAS, for many years, the United States Livestock Sanitary Association has provided the forum for the States, the Federal government, and Industry to get together and develop policies, procedures, and techniques for cooperative programs in the control and eradication of contagious and infectious diseases of livestock and poultry, and,

WHEREAS, this has been accomplished by the establishment of continuing standing committees with membership drawn from these groups, each one being assigned a specific sphere of interest and activity, and,

WHEREAS, generally speaking said standing committees have confined their interests to the development of procedures, policies, and the like, for cooperative programs affecting their assigned areas of interest, and,

WHEREAS, the matter of adequate financing, including the joint State-Federal funding and allocations of available monies for implementation and also general financial planning for eventual completion of projects, has been left largely to this Association's Committee on State-Federal Relations, and,

WHEREAS, the Committee on State-Federal Relations in its twice yearly meeting with United States Department of Agriculture people and others frequently finds itself inadequately informed on program financial needs or even plans and deadlines, so that its true effectiveness potential perhaps is seldom realized,

NOW THEREFORE BE IT RESOLVED by the United States Livestock Sanitary Association, assembled at Buffalo, New York, for its 1966 annual meeting, that:

I. Each standing committee, through its chairman, be charged with informing the chairman of the Committee on State-Federal Relations of the financial needs of its programs, from both a long-term and a short-term point of view, so that said State-Federal Relations Committee may be in a position more effectively to discharge its functions and duties.

II. This information be transmitted by each standing committee chairman to the chairman of the Committee on State-Federal Relations within the sixty-day period immediately following the adjournment of each regular annual meeting of the United States Livestock Sanitary Association.
III. Such financial statements be made a part of each committee's annual report to this Association.

SHEEP AND CATTLE SCABIES

Proposed by Committee on Parasitic Diseases and Parasiticides.

WHEREAS, the accelerated sheep scabies eradication program has progressed to the point that the incidence of the disease has been reduced to its lowest level in the Association's history and

WHEREAS, the goal of complete eradication of the disease is yet to be reached

THEREFORE, BE IT RESOLVED, that this Association and all regulatory officials exert every effort this winter to conduct an eradication program designed to complete the eradication of the disease.

WHEREAS, there has been an increase in the incidence of cattle scabies, and

WHEREAS, there is a need to place continued emphasis on scabies inspection activities particularly in areas involved in last years outbreak and areas where foci of the disease may still exist,

THEREFORE, BE IT RESOLVED, that this Association support extended inspection activities designed to locate and eliminate cattle scabies.

RABIES

WHEREAS, rabies is and has been a serious problem of long standing in the United States, as well as the world; and,

WHEREAS, the occurrence of rabies is a serious human health threat and a continuing threat to all warm blooded animals and a costly disease both in animal losses, as well as requiring costly control measures; and,

WHEREAS, rabies has been dealt with sometimes separately and sometimes jointly by various agencies, organizations and individuals by a wide range of leadership; and,

WHEREAS, measures and approaches applied are varied in various localities with varying degrees of effectiveness; now, therefore,

BE IT RESOLVED, that the United States Livestock Sanitary Association immediately and vigorously promote the formation of a National Rabies Council which will bring under one group representatives from all other groups which are engaged in rabies investigation. It shall be the function of the National Rabies Council to gather together all information pertinent to the rabies problem. This Council will bring together those individuals who are knowledgeable in rabies control, research and
epidemiology to make recommendations for the uniform control and ultimate eradication of rabies. This Council may wish to consider proposed legislation at the National, State and local levels.

INTRAUTERINE INFECTIONS

WHEREAS, the problems associated with Intrauterine infections and embryo abnormalities in swine are receiving greater recognition from the swine industry and veterinary scientists; and

WHEREAS, in the interest of scientific exchange between groups working in various phases of this general problem, it is deemed necessary to coordinate and correlate present knowledge concerning these problems; and

WHEREAS, the Animal Health Division and Animal Disease and Parasite Research Division of the United States Department of Agriculture, in cooperation with the National Academy of Sciences, also recognizes these inherent problems; and

WHEREAS, the Committee on Transmissible Diseases of Swine of the United States Livestock Sanitary Association, through its discussions while assembled in session at this convention, feels the need of further clarifying those gray areas which remain unsolved in connection with intrauterine health; NOW, THEREFORE,

BE IT RESOLVED That the United States Livestock Sanitary Association assembled in Convention this 14th day of October, 1966, does hereby commend and endorse the proposed Symposium on Intrauterine Infections and Embryo Abnormalities in Swine to be held in April, 1967, and it is anticipated that such interchange as is developed at this meeting will stimulate new approaches to the problems associated herein in those areas wherein research is presently lacking.
REPORT OF THE COMMITTEE ON STATE-FEDERAL RELATIONS

G. S. Kaley, Albany, New York, Chairman; W. L. Bendix, Richmond, Virginia; J. G. Flint, Minneapolis, Minnesota; R. A. Hendershott, Trenton, New Jersey; J. L. O’Harra, Reno, Nevada; J. F. Quinn, Lansing, Michigan; J. C. Shook, Harrisburg, Pennsylvania

The contacts between your Committee and the representatives of the Agricultural Research Service during 1966 were influenced in marked degree by the apparent determination of the federal administration to curtail expenditures for selected domestic programs in favor of other commitments.

As early as January 24, in a telegram to cooperating state officials, it was made known that initial federal budget projections for the 1967 fiscal year called for a reduction of $1,702,100 in brucellosis funds and a decrease of $484,300 in the amount earmarked for scabies eradication. Later estimates modified these figures to $1,583,400 and $472,100 respectively. The scabies programs in the individual states would be affected in varying degree depending on the disease situation in the state. Individual state allotment of brucellosis funds would be reduced in the case of any state which had less than one percent infection for two years and which imported only from states with a similar or lesser incidence of infection.

It seemed clear to your Committee that these figures were firm and not subject to substantial adjustment or change by the Agricultural Research Service. In view of this President Campbell took the matter of the 1967 budget directly to the Congress in a statement made before the respective Subcommittees on Agricultural appropriations of the Senate and House of Representatives. I quote from his presentation those portions which deal with Brucellosis and scabies:

"BRUCELLOSIS - Presently, about 90% of the nation is modified certified or brucellosis free. As a result, a mandate was set down just prior to the turn of this year by these states that unless the 10% of the states which were lagging had attained a like status by January 1, 1968, they would be unable to ship their cattle into these free or relatively free areas. This action has done more during the past four months to stimulate activity in these states which have not been taking a positive course to eradicate the disease than all other factors combined over the last five years.

This increased desire, however, places quite a financial burden on the Agricultural Research Service to cooperate with these states as fully as they now could, particularly in view of the approximate 1 3/4 million dollar presidential reduction in brucellosis funds for Fiscal Year 1967, coupled with the 60-40 federal to state matching fund limitations imposed on the Department.
We now have the necessary impetus to accomplish our goal of eradication, and in the opinion of the United States Livestock Sanitary Association, this is a most inopportune time for a reduction in brucellosis funds. On the contrary, should the Congress see fit to provide funding as was originally requested in this budget and on a contingency basis remove the 60-40 matching ratio limitation in those states which have evidenced this intensified desire to "get in line" with the rest of the nation, we feel that all of the states can achieve a modified certified status by 1969.

SHEEP AND CATTLE SCABIES - Two years ago we advised the Congress through your Committee that should it see fit to provide funds for the eradication of sheep scabies from the United States at a level of one and one-half million dollars annually for a three year period, from that point onward a quarter of a million dollars per year would be adequate for keeping the disease out of the country. The Congress judiciously followed this recommendation for two of these three years, and the disease has been dramatically reduced to the point where financing on the same level for this third year would see its elimination as predicted. However, if the half million dollar cut in this program as contemplated by the Administration is approved this year, sheep scabies will regain the foothold which it had throughout the country two years ago and we can expect a never-ending expenditure for control.

We would emphasize an allotment at the previously recommended level of one and one-half million dollars for this year."

Dr. Campbell's statement related to the needs of 1967. His words will be equally valid in 1968. The following table provides information on the long range views of your Committee on the 1968 budget. The figures are of course subject to such modification as develops during the coming months may dictate.

Several days ago we were informed that the 1967 Agricultural Research Service budget bill, as finally approved early in September, provide funds in excess of the requests shown in Table I for work on a number of diseases.

In connection with federal financing in general, it is the view of this Committee that the privilege of transferring at least 10% of available funds from one program to another, once enjoyed by the Animal Health Division, should be restored. The present policy of rigid adherence to the letter of each appropriation act allows for no adjustment to unforseen or unpredictable circumstances.

You will note in the table our recommendation that the so-called 60/40 rule be waived to permit concentration of effort in those states which have not yet achieved the modified-certified status. They number some 12 as this is written. While it might be argued that many of the states that have recently reached that goal have done so with the 60/40 rule in effect, it is obvious that a rapid reduction in the incidence of Brucellosis in the remaining 12 states is in the best interest of every other state. It would seem that this can best be done by concentrating efforts in those areas where the most remains to be done.

Tuberculosis. With regard to tuberculosis, a statistical projection
## TABLE I - Animal Health Division Appropriations

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<td></td>
<td></td>
<td></td>
<td></td>
<td>Remarks</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>$</td>
<td>$</td>
<td>$21,071,700</td>
<td>$19,488,300</td>
<td>$19,488,300</td>
<td>No reduction from 1967 plus waiver of 60/40 rule</td>
<td></td>
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<tr>
<td>Tuberculosis</td>
<td></td>
<td></td>
<td>3,229,000</td>
<td>3,252,800</td>
<td>4,252,800</td>
<td>Increase of $1,000,000 to shorten eradication period</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$23,003,168</td>
<td>$23,958,300</td>
<td>$24,300,700</td>
<td>$22,741,100</td>
<td>$23,741,100</td>
<td></td>
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<tr>
<td>Sheep Scabies</td>
<td></td>
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<tr>
<td>Cattle Scabies</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>$655,400</td>
<td>$1,476,800</td>
<td>$1,519,600</td>
<td>$1,047,500</td>
<td>$1,297,500</td>
<td>Restoration of $250,000 of last years $472,100 cut</td>
<td></td>
</tr>
<tr>
<td>Hog Cholera</td>
<td>$3,486,300</td>
<td>$3,615,500</td>
<td>$4,431,100</td>
<td>$4,464,300</td>
<td>$5,064,300</td>
<td>An additional $600,000 will be needed for indemnity</td>
<td></td>
</tr>
<tr>
<td>Cattle Ticks</td>
<td>701,500</td>
<td>741,100</td>
<td>757,200</td>
<td>763,400</td>
<td>833,400</td>
<td>$70,000 needed to expand area of inspection &amp; for National Tick Survey</td>
<td></td>
</tr>
<tr>
<td>Screwworm</td>
<td>2,750,000</td>
<td>3,456,200</td>
<td>4,158,400</td>
<td>3,848,400</td>
<td>5,348,000</td>
<td>$1,500,000 will permit extending the eradication zone into Mexico</td>
<td></td>
</tr>
<tr>
<td>Interstate Movement</td>
<td>2,819,000</td>
<td>2,301,500</td>
<td>2,356,600</td>
<td>2,379,000</td>
<td>2,379,000</td>
<td>Maintain at current level</td>
<td></td>
</tr>
<tr>
<td>Inspection and Quarantine</td>
<td>1,354,018</td>
<td>1,607,700</td>
<td>1,815,800</td>
<td>1,930,200</td>
<td>2,330,200</td>
<td>Shore up personnel for better surveillance over prohibited meats</td>
<td></td>
</tr>
<tr>
<td>Capital Construction</td>
<td></td>
<td></td>
<td>100,000</td>
<td></td>
<td>850,000</td>
<td>This amount plus other available funds should replace the Clifton facility</td>
<td></td>
</tr>
<tr>
<td>Poultry Disease</td>
<td></td>
<td></td>
<td>1,500,000</td>
<td></td>
<td></td>
<td>Set up poultry disease as a separate budget item</td>
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<tr>
<td>Miscellaneous Diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1. Trichinosis</td>
<td></td>
<td></td>
<td>1. +100,000</td>
<td></td>
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</tr>
<tr>
<td>2. Vesicular Stomatitis</td>
<td></td>
<td></td>
<td>2. +100,000</td>
<td></td>
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<td></td>
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<tr>
<td>3. E.L.A. &amp; piroplasmosis</td>
<td></td>
<td></td>
<td>3. +150,000</td>
<td></td>
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<tr>
<td>4. Scrapple</td>
<td></td>
<td></td>
<td>4. +150,000</td>
<td></td>
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<tr>
<td>5. Foot Rot</td>
<td></td>
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<td>5.</td>
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<tr>
<td>6. Cattle Grubs</td>
<td></td>
<td></td>
<td>6. +50,000</td>
<td></td>
<td></td>
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<tr>
<td>7. Ram Epididymitis</td>
<td></td>
<td></td>
<td>7.</td>
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</tr>
<tr>
<td>8. F.A.O.</td>
<td></td>
<td></td>
<td>8. +100,000</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>$849,500</td>
<td>$891,400</td>
<td>$905,900</td>
<td>$1,217,100</td>
<td>$1,867,100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aYear ending June 30; bStill before Congress 9/1/66; cClifton Quarantine Station plans; dForeign Animal observation.
indicates that at the present rate of progress, total eradication can be achieved by 2006. Federal expenditures for this program approximate $3,000,000 per year while the states contribute about $9,000,000. It is estimated that if the federal contribution to this program were increased by $1,000,000 per year, eradication could be achieved by 1986. The net saving to the federal government should be in the neighborhood of $40,000,000.

Research. Your Committee also gave thought to the matter of research needs. If our livestock industry is to continue to meet the needs of a constantly expanding population, it is essential that a high level of efficient productivity be maintained in our flocks and herds.

The development of superior types through applied genetics has already brought us to a point where increased production per bird or animal can be counted upon to satisfy only a fraction of the increased demand.

There remain two ways in which the total output of animal proteins can be substantially increased. One is to raise more animals. The second is to reduce the very substantial losses caused by disease including infertility and parasitism.

Our ability to control disease can, in turn, be developed to its full potential only through research.

Since our border defenses, in the light of today's high speed transportation, are meager at best, it is imperative that plans and procedures for containment and eradication of infectious diseases, foreign as well as domestic, be developed and maintained in state of constant readiness.

It is recommended that funds be provided for additional animal quarters at Plum Island and that assignment of staff veterinarians to stations abroad for field training in the diagnosis and epidemiology of exotic diseases be expanded. The knowledge thus gained will be invaluable in suppression of those diseases which do gain a foothold in North America. Lack of adequate preparation in these areas could be catastrophic. Much also remains to be learned about a number of native diseases.

Biologics. Over the years this Committee has repeatedly called attention to the hazards inherent in the licensing and distribution of untested or inadequately tested biological products. In the light of the poultry vaccine problems that have arisen in the last year, some of these warnings were akin to the prophetic. As the presentation by Drs. Pomeroy and Newman on Wednesday suggests, this is not an area in which the individual states can take the leading role. At the risk of belaboring the point, we once more urge that the Agricultural Research Service make the strongest representations to the administration and to the Congress in behalf of realistic financing of the Biologics Division.

Poultry Diseases. With respect to poultry diseases, the United States Livestock Sanitary Association, for some years, through this Committee, has been requesting the Department to recognize the importance of the commercial poultry industry by greatly increasing its cooperative disease-control efforts in this field and setting up its regulatory budget for poultry diseases as a separate-line item.

Commercial poultry operations in the United States have grown to
sufficient size to rival the swine industry in dollar volume. Insofar as its potential for the production of high-protein food in a short period of time is concerned, this industry has no rival. We are told that the food surpluses which have plagued us for many years are either rapidly disappearing or, in some commodities, have disappeared. In the face of an exploding national population, we are or shortly will be faced with a demand for considerably increased food production, and we will turn increasingly to the poultry industry to do its share. If you will relate this situation for a moment to food conditions and needs existing around the world, you will see that our responsibility in this field represents not only an enormous challenge but an acute threat to peace. It has been said that men do not starve quietly and peacefully, and that they are even less inclined to see their families do so. In the face of an exploding world population, particularly in those areas least able by climate, soils, and technology to feed their existing populations, the role of the United States will be an increasingly important one. It will also be vital to our own interests and safety fully to discharge our obligations in this field. Other than perhaps some salt-water or oceanic supply of high-quality protein food, what other source presents such a potential as poultry? It will fill not only a short-term need for exporting high-quality protein food in large volume because of our ability for rapid expansion, but it possesses enormous potential as an exportable industry that can rapidly be developed to fulfill the same needs in other parts of the world.

Before this can be done, however, we must solve or at least begin to control and eradicate some of the serious disease problems with which the poultry industry is plagued. Commercial poultry operations have grown so fast and become so large that their disease problems have outgrown even our own knowledge of them, and our specific knowledge in control and eradication is still inadequate. To add to this problem, communications between the main segments of the poultry industry and the regulatory disease-control services of organized veterinary staffs of the State and Federal governments have not been good. The only real disease-control effort the industry has undertaken is strictly a voluntary one, basically in the hands of laymen. This is not in any sense meant to be critical, because the pullorum-typhoid control program of the National Poultry and Turkey Improvement Plans has done a magnificent job. The fact does remain, however, that it has been a voluntary program, and there still are some few centers of infection that need to be eliminated, by mandate. In addition, there are other Salmonellae coming along that no doubt will require professional attention. The problem of leukosis is not solved, and it is a cause of major losses. Newcastle disease is not behind us, nor are laryngotracheitis, bronchitis, and others. Mycoplasma infections, which have contributed perhaps the highest percentage of condemnations in commercial slaughter, we are only now beginning to provide some relief, and still on a voluntary basis. Even now we do not know whether we are heading toward a testing program similar to the pullorum-typhoid effort or whether we are heading toward some form of vaccination—or perhaps a combination of both.
There are other problems that need attention before this industry can provide us with the enormous supplies of valuable protein that this world is going to need, and shortly. It is only after we begin to control these things that the poultry industry's potential can be realized, both at home and abroad. Because of this, we again ask and urge the Department to provide 1.5 million dollars as a line item for the control of poultry diseases and to actively inaugurate cooperative poultry-disease control programs in the twenty odd states of this nation where the poultry industry represents an important segment of their economy. We badly need not only the impetus and drive that such a Federal effort would provide, but we need to begin to develop uniformity in our approach, so that we can develop within this industry the kind of confidence necessary for a truly cooperative effort that will produce results.
REPORT OF THE COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION


The Committee briefly reviewed last year's report in reference to two items referred for further study. One item—feasability study of establishing specific approval of markets under one set of requirements instead of the several now being used was reported by Dr. Fred Hanson, Jr., of the Stockyards Section of ARS., AN.H. He said the section had done considerable study on this matter and is now exploring the legal problems in such a change. He reported that they will continue the study to a conclusion, but some of the basic standards need to be further defined, considered and discussed with marketing agencies before final recommendations can be made. The other matter—a recommendation that the state and federal animal health officials review the status of specifically approved markets within their individual states with the suggested removal of specific approval from markets which no longer have a need for such status. The Committee feels that most states have followed the recommendations in the markets approved for movement of swine under part 76 but wishes to again encourage review relative to other specific approval status.

Dr. Don Johnson of AN.H. emergency disease staff was asked to review with the Committee the effect upon marketing of livestock that might be felt in the event that an outbreak of foreign disease (such as foot and mouth disease) should occur involving one or more markets in the United States. It was the consensus that awareness of the problem by market operators and others is necessary. The degree of apathy, along with the increasingly rapid movement of livestock makes the fears of animal health officials more realistic. The Committee agreed that in such an event readily available records at markets accurately depicting movements with minimum interference of marketing procedures and operations. Good records would likely shorten the length of time that curtailments of movement might be necessary in order to control the outbreak. The Committee offers assistance to Doctor Johnson and the Committee on Foreign Animal Diseases in establishing a basic plan or guideline to help to avoid panic and undue curtailment of livestock movements in such an eventuality.

The Committee also urges each state to re-evaluate its record keeping at markets to determine that such information on movements will always be rapidly available. It was noted that other subjects being considered by the Committee were in line with the same problem to a great
extent, namely, identification of market animals; and market construction that lends itself readily to adequate cleaning and disinfection.

A set of guidelines or basic criteria for facility standards of a model livestock market, having been previously submitted to all members of the Committee by mail, were reviewed. The Committee, in hopes of developing acceptable standards for a model market felt that facility standards are of basic importance to further progress in this area. The chairman was asked to re-draft the guidelines with incorporation of the various suggested changes and re-submit them to the Committee members soon for further review in depth. It is expected that sufficient review and study of these corrected guidelines can be accomplished this year allowing for submission to the United States Livestock Sanitary Association for adoption at the meeting in Phoenix next fall.

It was recognized that market operators have already taken the lead in some areas to upgrade their facilities and systems of operation, and should also take an active part in developing further guidelines for others to follow.

The Committee recognized with considerable enthusiasm the work of the stockyards section of AN.H. in their fulfillment of numerous United States Livestock Sanitary Association requests in replacing the much discussed form '48 with the new AN.H. form 2-7. AN.H. form 207 simulates the Uniform Health Certificate form previously adopted by the United States Livestock Sanitary Association and makes obsolete the present AN.H. forms 2-12, 2-24, 2-24A, 2-24B, 2-48, 2-48A, and 2-48B, all of which will very soon be completely out of use.

Correspondence to the Committee from Independent Livestock Marketing Association was read and acknowledged at our committee meeting here in Buffalo. Thanks were also expressed to the Certified Livestock Markets Association for their sponsorship of the Livestock Health Coordination Conference and for our committee's participation therein.

Review of the committee's membership was given and suggestions to upgrade committee structure were forthcoming.

Our committee meeting here in Buffalo was adjourned to re-open immediately as a joint meeting with the Committee on Laws and Regulations. Correspondence from Dr. J. Flint as a portion #3 of last year's report from the Committee on Tuberculosis and Paratuberculosis was read before the joint committee. This request from the Tuberculosis committee to the Stockyards committee along with other suggestions to the Laws and Regulations committee last year, culminated in a joint sub-committee being established from membership of the two committees. The sub-committee report was delivered by its chairman, Dr. Fred Hanson, Jr., and was adopted by the two committees meeting jointly on Tuesday. As portrayed to you in the report of the Committee on Laws and Regulations yesterday morning, it was decided that the sub-committee report should be included in the report of the Committee on Stockyards, Markets and Transportation. The following, then, is the report of the joint sub-committee on Animal Identification.
Dr. F. W. Hanson, Jr., *Chairman*; Dr. T. A. Ladson, Dr. D. Smith, Dr. I. Erickson, representing the Committees on Stockyards, markets and Transportation and Laws and Regulations. Committee meeting Oct. 10, 1966 at Buffalo, New York. Others in attendance were Dr. D. A. McGill, and Dr. R. C. Sexauer.

While the Committee realized that the scope of the area was not limited strictly to the identification of cattle, nor to the use of backtags, it did, in large part, address itself to this phase of the activity. Specific items reviewed included:

1. Maintaining the identity of cattle handled by dealers, generally, it was agreed that the identity of cattle handled by dealers was often obscured or lost, making tracebacks difficult if not impossible. Removal of backtags by dealers is a problem in some areas. The success, or lack of it, in states having dealer regulations were reviewed.

2. Direct marketing. The trend is toward more shipments direct from farm or ranch to packer through order buyers, buying stations and plant deliveries. To a large extent such animals are not adequately identified for traceback purposes.

3. The use of ear tags. Some states do not use a system wherein ear tags can be readily correlated with the herd in which the tag was applied. Ear tags do not necessarily denote the herd of origin. With expansion of screening procedures (B.R.T.*M.C.T.) the number of ear tags applied will steadily diminish.

4. There is a need for a system to identify animals in trade channels that are unfit for breeding or feeding purposes.

5. T.B. lesion cases found on regular kill of feedlot cattle are particularly difficult to trace.

6. Blood sample recovery rate from backtagged cattle fluctuates between different areas and time periods. The national average is 59 percent. Steps need to be taken to increase and stabilize these returns.

The Committee feels that one area most in need of attention is the dealer problem. It is suggested that intrastate identification requirements be furthered in states not now regulating this area. The Committee understands that 14 states now have such requirements for livestock dealers which include that the identity of animals handled be maintained.

Companion to this need, the Committee recognizes the need for an interstate identification regulation and recommends that the AN.H. Division be asked to develop such regulations. At the present time, the Committee feels this proposal should apply only to slaughter cows over two years of age; to be identified by backtag, or a brand that is recognized for official brand inspection purposes. The Committee feels that any such regulation of this nature must have wide review with industry, state officials and other interested groups prior to promulgation.
The Committee also urges that states not now doing so, install a record keeping system for correlating ear tags with herds of origin. The Committee suggested that ADE 1-62 report or some similar system would accomplish this purpose.

Gentlemen, this concludes the report of the Committee on Stockyards, Markets and Transportation. I respectfully request that it be submitted to the executive committee of the United States Livestock Sanitary Association and urge its adoption.
REPORT OF THE COMMITTEE ON ANIMAL VIRTUAL CHARACTERIZATION


The activities of the Committee’s virus characterization data collection and processing center, at the Institute for Comparative Biology, San Diego are continuing under the guidance of Dr. C. J. York, with the continued financial support of the National Institutes of Health.

During the past year, two workshop-type conferences have been held, one at San Diego, California and the other during these sessions. The primary purpose of these conferences was to examine and evaluate the characterization data on approximately 130 viruses. From this group the Committee has selected 42 strains that can be considered sufficiently studied to be designated as reference viruses. The list of these proposed reference viruses has been submitted to and approved by the Eastern Hemisphere representatives. A recommendation will be made to the American Type Culture Collection to procure, deposit and make available the viruses designated as reference strains. The selected lists of reference viruses will be published in a supplementary report by the San Diego Center.

The Committee has also undertaken the revision of the virus characterization data questionnaire form to make it more adaptable to a data processing system. This will facilitate the achievement of one of our goals, that of assembling a catalogue of animal virus characterization data.

With the selection of certain reference viruses a way is now open to begin the preparation of reference reagents. Considerable time has been devoted by the Committee to the establishment of standards for the preparation of such reagents and to the ways and means of supporting these vital activities.

The Committee would like to emphasize the importance and the existing need for reference antiserums and antigens which are essential for the definitive identification of indigenous animal viruses. Since such reagents have direct bearing on animal and human health, the Committee urges the United States Department of Agriculture and the United States...
Public Health Service to enlarge their support for development, production, and distribution of such reagents.

The Committee would also like to indicate that studies in the field of microbiological disease agents, including viruses, are providing ever-increasing evidence on the existence of antigenic and immunologic similarities between agents, irrespective of animal or geographical origin. Thorough understanding of such cross-relationships is essential for accurate characterization and identification of viruses indigenous to this country. In order to achieve these ends, there is an urgent need for viral antiserums not now available to research workers in the United States. The Committee, therefore, recommends that the United States Department of Agriculture cooperate in making available for research purposes a stock of specific antiserums for certain animal disease agents foreign to this country. It is proposed that qualified investigators, having appropriate laboratory facilities, be provided with limited amounts of such antiserums for specific research purposes.

When the deliberations of the Committee on the standards for production of reference reagents are completed, the Committee's recommendations will be published.

An important function of this Committee is the dissemination of basic and applied virological data. To this end, Committee members have participated in the organization and presentation of symposia in various fields of virology. An example of such a symposium was one held at the 1966 meeting of the American Veterinary Medical Association. The proceedings of this symposium will be published in the American Journal of Veterinary Research.

One of the major aims of the Committee is to make available virus characterization data to international bodies concerned with the definitive classification of viruses and the development of virus nomenclature systems. The recent appointment of Dr. Charles J. York to the membership of the International Committee on the Nomenclature of Viruses, created this year by the International Societies of Microbiology, and his participation as one of the United States representatives in conferences on virus nomenclature held last July in Moscow have contributed greatly to the fulfillment of the Committee's aims.

In order to bring into our activities broader participation of virologists from foreign countries, a number of colleagues from Canada, Latin America, Japan and Australia have been invited to participate in our work as corresponding members.
A HEMAGGLUTINATION-INHIBITION TEST FOR PARAINFLUENZA 3 VIRUS ANTIBODIES

G. H. Frank, D.V.M., M.S., Ph.D.

Ames, Iowa

REVIEW OF LITERATURE

Parainfluenza 3 (PI-3) virus, which is involved in shipping fever of cattle, has the ability to agglutinate human O, guinea pig, bovine, and some fowl erythrocytes (RBC). Serologic tests for antibody to this virus include the hemagglutination inhibition (HI), serum neutralization (SN), and complement fixation (CF) tests. The HI test is the simplest of these, and is widely used. It is very reproducible, and HI titers have been found to parallel SN titers. Among the various workers, methods of the HI tests differ on several points. Volumes of serum dilution, virus suspension, and RBC suspension, concentration and kind of RBC, and incubation times and temperatures are points of difference; otherwise, tests are similar. However, there is marked disagreement among workers as to whether or not it is necessary to treat serums to remove nonspecific inhibitors of hemagglutination (HA). Reisinger et al. found untreated bovine serums to have nonspecific HI titers which could be lowered by treatment with receptor destroying enzyme (RDE). Clarke and Casals, working with anthropod-borne viruses, treated human, monkey, mouse, guinea pig, rabbit, and chicken serums with a 25 percent suspension of acid-washed kaolin to remove nonspecific HA inhibitors. Kaolin treatment, sometimes with certain modifications, was also used by Rosen in working with adenoviruses, and by Hoerlein et al., Abinanti et al., Gale and King, Woods et al., Heddleston et al., Kramer et al., and Saunders et al. in working with bovine serums and PI-3 virus. Chanock et al., working with parainfluenza viruses, treated human serums with kaolin.

Bakos and Dinter questioned the value of treating bovine serums with RDE, since they found no differences in the titers of untreated and RDE-treated serums. Ketler et al. tested for the presence of nonspecific HA inhibitors of parainfluenza 1 and 3 viruses in human, rabbit, and bovine serums, and concluded that they were either absent or of such low titer to be of no consequence. Dawson used a wide range of techniques to remove nonspecific inhibitors from bovine serums and define them. However, he found no evidence of such inhibitors and considered treatment of bovine serums prior to examination for PI-3 virus antibodies unnecessary.

Most workers in this area have treated serum samples by RBC adsorption to adsorb out nonspecific hemagglutinins. From the Bacterial and Mycotic Diseases Investigations, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Ames, Iowa.
The RBC used for adsorption were from the same species as those used in the HI tests.

In this study, a HI test for antibodies to PI-3 virus in bovine serum, and a method for treatment of serums are described and evaluated.

**MATERIALS AND METHODS**

**Serums.** Thirty seven serum samples were obtained from colostrum-deprived, isolation-raised calves ranging from one day to eight months in age. These calves had no known contact with PI-3 virus. Serum samples were also obtained from some of these calves after exposure to PI-3 virus. Samples were stored at -20 C or -70 C until tested.

**Virus.** The SF-4 strain of PI-3 virus, isolated by Reisinger et al. was used. It had undergone 10 passages in bovine embryo kidney tissue culture. Virus for the HI test was grown in bovine embryo kidney tissue culture, harvested after 72 to 100 hours, and stored at -70 C until used.

**Erythrocytes.** Heparinized samples of bovine blood were washed three times in 0.85 percent NaCl in distilled water (saline). On the final washing, the RBC were packed by centrifugation at 400 x G for 15 minutes, then were reconstituted to a one to four suspension in saline and stored at 4 C. Erythrocyte suspensions were discarded after four days.

**Hemagglutination (HA) Test.** Tubes containing 0.5 ml of twofold serial dilutions of virus in saline were mixed with 0.25 ml of 0.42 percent packed bovine RBC. Tests were held at 4 C overnight before they were read. The highest virus dilution causing complete HA was stated to contain one HA unit of virus per 0.5 ml.

**Treatment of Serums.** Bovine serums were diluted one to two in saline, and approximately 0.1 Gm of acid-washed kaolin* was added with a spatula to 1.2 ml of the serum dilution. After 10 minutes at room temperature, with occasional shaking, the kaolin was removed by centrifugation at 400 x G for 10 minutes. After kaolin adsorption, 0.5 ml of serum dilution was placed in a water bath at 56 C for 30 minutes.

**Hemagglutination Inhibition (HI) Test.** Serums were diluted twofold in saline, the first dilution being one to two. Volumes were 0.25 ml. To this, 0.25 ml of virus suspension containing four HA units was added to each tube. After incubation for one hr. at room temperature, 0.25 ml of RBC suspension, containing 0.42 percent packed bovine RBC, was added to each tube. Tests were held at 4 C overnight before they were read. A saline control and serum controls were included. Titers were expressed as reciprocal of the initial serum dilution in the last positive tube, and designated + if the next tube showed incomplete HI.

**Experimental Variations of the HI Test.** Several variations in the test were included to show the presence of nonspecific HA inhibitors and to show the effects of certain changes in methods and materials on the test.

*Fisher Scientific Co., Fairlawn, N. J.*
A HI TEST FOR PI-3 VIRUS ANTIBODIES

To show the presence of nonspecific HA inhibitors, the pre-exposure serums were tested untreated, and after incubation at 56°C for 30 minutes. Tests were also run with two HA units of virus per 0.25 ml and with RBC obtained from cattle that were from three weeks to 12 1/2 years in age. Effect of time in incubation of the virus and serum before addition of RBC also was studied.

RESULTS

Hemagglutination inhibition titers on serums from 37 colostrum-deprived, isolation-raised calves before and after heat treatment of samples, after heat and kaolin treatment, and against two and four HA units of virus are shown in Table I. Inhibitors of the HA activity of PI-3 virus were found to be present in untreated serums, and usually to a lesser degree in heat-treated serums. When adsorption with kaolin was included, "titers" were further reduced. Inhibitors could be demonstrated in greater dilutions of the serums when less virus was used in the test.

Table II shows the results of HI tests on 21 serums from colostrum-deprived, isolation-raised calves which had been exposed to PI-3 virus. Serums were tested before treatment and after heat and kaolin treatment with four HA units of virus. As shown, treatment of serums did not significantly reduce actual titers.

The effect of incubation time of the virus-serum mixture before addition of RBC is shown in Table III. One serum of high titer and one of intermediate titer were tested. The RBC suspension was added from 0 to 90 minutes after virus had been added to the serum dilutions.

At least 30 minutes' incubation of the virus-serum mixture was necessary for a serum of high titer to exert its full inhibitory effect on the virus.

HI tests were run on three serum samples, one negative, one of intermediate titer, and one of high titer, using RBC from 11 different cattle that were from three weeks to 12 1/2 years of age. There were no differences in the resulting titers.

Tests on heat-treated serums were irregular and endpoints were vague. Irregular-bordered buttons of RBC were common. This occurred to much less extent with untreated serums, and seldom with kaolin adsorbed serums. Tests on kaolin adsorbed serums were clear cut and endpoints were definite.

Serum control tubes were included with each test. No bovine serum sample caused spontaneous agglutination of bovine RBC.

DISCUSSION

As presented in Table I, there are substances in serums of colostrum-deprived, isolation-raised calves, which will inhibit HA of bovine RBC by PI-3 virus. The inhibitor was not present in all calf serums tested, and the levels varied from calf to calf. "Titers" of untreated serums ranged from 2+ to 16 when 4 HA units of virus were used in the test. Heating the serum at 56°C for 30 minutes usually reduced the "titer" somewhat.
Hemagglutination Inhibition Titers\(^a\) of Colostrum-Deprived, Isolation-Raised Calves to Parainfluenza 3 Virus

<table>
<thead>
<tr>
<th>Calf Age</th>
<th>2 HA Units of Virus Used</th>
<th>4 HA Units of Virus Used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{A}^b)</td>
<td>(\text{B}^c)</td>
</tr>
<tr>
<td>5403</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5406</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5466</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5468</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5469</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5475</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5480</td>
<td>2 da.</td>
<td>4</td>
</tr>
<tr>
<td>5567</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5569</td>
<td>1 da.</td>
<td>4</td>
</tr>
<tr>
<td>5571</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5573</td>
<td>1 da.</td>
<td>8</td>
</tr>
<tr>
<td>5565</td>
<td>2 mo.</td>
<td>8(^+)</td>
</tr>
<tr>
<td>5566</td>
<td>2 mo.</td>
<td>2(^+)</td>
</tr>
<tr>
<td>5568</td>
<td>2 mo.</td>
<td>2(^+)</td>
</tr>
<tr>
<td>5572</td>
<td>2 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5511</td>
<td>3.5 mo.</td>
<td>8(^+)</td>
</tr>
<tr>
<td>5512</td>
<td>3.5 mo.</td>
<td>8</td>
</tr>
<tr>
<td>5501</td>
<td>4 mo.</td>
<td>16</td>
</tr>
<tr>
<td>5506</td>
<td>4 mo.</td>
<td>8</td>
</tr>
<tr>
<td>5507</td>
<td>4 mo.</td>
<td>8</td>
</tr>
<tr>
<td>5576</td>
<td>4 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5577</td>
<td>4 mo.</td>
<td>8(^+)</td>
</tr>
<tr>
<td>5394</td>
<td>5 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5397</td>
<td>5 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5400</td>
<td>5 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5401</td>
<td>5 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5409</td>
<td>5 mo.</td>
<td>16(+)</td>
</tr>
<tr>
<td>5412</td>
<td>5 mo.</td>
<td>16(+)</td>
</tr>
<tr>
<td>5494</td>
<td>5 mo.</td>
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</tr>
<tr>
<td>5496</td>
<td>5 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5136</td>
<td>5.5 mo.</td>
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</tr>
<tr>
<td>5477</td>
<td>6 mo.</td>
<td>16</td>
</tr>
<tr>
<td>5478</td>
<td>6 mo.</td>
<td>32</td>
</tr>
<tr>
<td>5128</td>
<td>8 mo.</td>
<td>8(+)</td>
</tr>
<tr>
<td>5134</td>
<td>8 mo.</td>
<td>8(+)</td>
</tr>
<tr>
<td>5139</td>
<td>8 mo.</td>
<td>8(+)</td>
</tr>
<tr>
<td>5479</td>
<td>8 mo.</td>
<td>32</td>
</tr>
</tbody>
</table>

\(^a\)Titers are expressed as the reciprocal of the initial serum dilution of the last positive tube, and designated as + if the next tube is incomplete.

\(^b\)Untreated serum.

\(^c\)Heat-treated serum.

\(^d\)Kaolin-adsorbed, heat-treated serum.

However, heat treatment caused the tests to be difficult to interpret. When adsorption with kaolin was included, the "titers" were further reduced, and tests were clear cut and more easily interpreted. By using two HA units of virus, the test was made more sensitive, and inhibitor could be demonstrated at higher serum dilutions.
TABLE II

Hemagglutination Inhibition Titers* of Colostrum-Deprived, Isolation-Raised Calves After Exposure to Parainfluenza 3 Virus

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Untreated</th>
<th>Kaolin-Adsorbed, Heat-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>16+</td>
</tr>
<tr>
<td>5</td>
<td>16+</td>
<td>16+</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>32+</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>12</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>13</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>14</td>
<td>256</td>
<td>256</td>
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<tr>
<td>15</td>
<td>256</td>
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<tr>
<td>16</td>
<td>256</td>
<td>256</td>
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<td>17</td>
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<td>19</td>
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<td>256</td>
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<tr>
<td>20</td>
<td>512</td>
<td>1024</td>
</tr>
<tr>
<td>21</td>
<td>512</td>
<td>512</td>
</tr>
</tbody>
</table>

*Titers are expressed as the reciprocal of the initial serum dilution of the last positive tube, and designated as + if the next tube is incomplete.

TABLE III

The Effect of Incubation Time of the Virus-Serum Mixture Before Addition of RBC on Hemagglutination-Inhibition Titer*

<table>
<thead>
<tr>
<th>Time of RBC Addition (minutes)</th>
<th>Serum of Intermediate Titer</th>
<th>Serum of High Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>128+</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>256</td>
</tr>
<tr>
<td>20</td>
<td>32</td>
<td>256</td>
</tr>
<tr>
<td>30</td>
<td>32</td>
<td>512</td>
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<tr>
<td>40</td>
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<td>50</td>
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<td>60</td>
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<td>512</td>
</tr>
<tr>
<td>70</td>
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<td>512</td>
</tr>
<tr>
<td>80</td>
<td>32+</td>
<td>512</td>
</tr>
<tr>
<td>90</td>
<td>64</td>
<td>512</td>
</tr>
</tbody>
</table>

*Titers are expressed as the reciprocal of the initial serum dilution of the last positive tube, and designated as + if the next tube is incomplete.

The fact that nonspecific inhibitors exist in bovine serums is supported by Reisinger et al. They reported that untreated serums had nonspecific HI titers as high as 64, which could be reduced to 16 or less.
by RDE treatment. Their serum titers probably were high because they used a 0.25 percent RBC suspension. Therefore, it would take less virus to comprise a HA unit, and if less virus were used, serum titers would appear higher.

Ketler et al.\textsuperscript{11} reported that nonspecific HA inhibitors were of no importance in bovine serums, and that titers were because of the presence of antibody. As evidence, they found 27 of 75 heat treated serums from healthy cattle and HI titers of less than 10. However, even though they used four HA units of virus, their suspension of packed RBC was 0.8 percent, which would result in their using more virus. This, along with pre-heating of the serum, may have resulted in their overlooking inhibitors when present in low concentration.

As other evidence, titers of serums from healthy cattle ranging from 20 to 640 were not reduced by RDE or kaolin treatment. Also, SN and HI titers on these samples closely agreed.

Bakos and Dink\textsuperscript{4} also reported that RDE treatment did not reduce serum titers.

Dawson\textsuperscript{7} found no nonspecific HA inhibitors in bovine serums, and also concluded that titers were because of the presence of antibody. As evidence, Dawson found three heated serums and one serum pool from colostrum-deprived calves to have HI titers of less than four. Titers of 20 serums (ranging from 8 to 512) from healthy cattle were not reduced by treatment with RDE, trypsin, periodate ion, kaolin, or bentonite. Also, he found a high degree of correlation between SN and HI titers. Upon fractionating serums, he found essentially all of the inhibitory substance in the gamma globulin fraction.

In this study, 12 of 37 heat treated colostrum-deprived calf serums had titers of four or less, five of these had titers of less than four. Kaolin adsorption and heat treating did not reduce titers of serum from colostrum-deprived, isolation-raised calves which had been exposed to PI-3 virus. Also, we have found HI and SN titer curves of positive serum samples to parallel each other, and pre-exposure serums with HI titers of four or less after kaolin and heat treatment to have no demonstrable neutralizing titers.\textsuperscript{13}

If a serum has a low HI titer, it is necessary to pretreat it to determine whether this was caused by nonspecific inhibitors. This study indicates that a serum titer of eight or more after treatment shows that the calf has come in contact with PI-3 virus. However, this is not a protective titer, since calves with HI titers of 32 were not protected when exposed to an aerosol of PI-3 virus.\textsuperscript{13}

Adsorption of bovine serums to remove nonspecific hemagglutinins was found to be unnecessary, since serum control tubes containing from 1:2 to 1:4 dilution of serum did not spontaneously agglutinate bovine RBC. For precautionary measures, however, a serum control should be included in each test.
A HI TEST FOR PI-3 VIRUS ANTIBODIES

SUMMARY

A HI test for determination of PI-3 virus antibodies in bovine serums is described. Treatment of serums by heat and kaolin was found to be necessary in order to remove nonspecific HA inhibitors. Bovine serums alone did not spontaneously agglutinate bovine RBC.

ACKNOWLEDGMENT

The author expresses his appreciation to Mr. L. C. Escher for his technical assistance.

REFERENCES


THE VITAL INTERESTS OF VARIOUS ORGANIZATIONS
IN VETERINARY BIOLOGICS

J. H. Gillespie*

A large number of veterinary organizations and groups have a vital interest in veterinary biologics. The availability of safe and efficacious biologics to control diseases of large and small animals is of great concern to all veterinarians; no matter what their role may be in our society. It is also important that our profession recognize the proper use and limitations of the various biologics available for use by the veterinary practitioner.

Three words are particularly important to my topic today—standards, understanding, and communications. To produce excellent veterinary biologics we must have a meaningful program of standards in veterinary microbiology. To establish standardized procedures requires a great deal of communication and of understanding between various groups of diverse interest and motivation. In particular, it requires the understanding, cooperation, and interest of control officials, practitioners, biological producers, laboratory diagnosticians, teachers, and researchers—individuals that represent practically all branches of the veterinary profession.

To produce excellent biologics it is necessary to develop standardized methods in veterinary microbiology. The idea to produce a manual (or manuals) of standardized methods in veterinary microbiology that would be useful to all veterinary groups originated in the AVMA Council of Biological and Therapeutic Agents four years ago. Our council at that time was asked by one of its consultants, Dr. John Hejl, to study the USDA Standard Requirements for Veterinary Biologics. It became apparent to the Committee on Biologics to the Council that there was a great need for additional standardized methods in veterinary microbiology and without them the task before them was difficult, if not impossible. In attempting to meet their objectives other organizations such as the Conference of Veterinary Laboratory Diagnosticians and the Western Hemisphere Committee on Non-primate Animal Virus Characterization as well as others, I am sure, have been aware of the great need to write standardized methods in veterinary microbiology covering such disciplines as animal virology, bacteriology, mycology, and possibly parasitology.

It was the feeling within the AVMA Council on Biological and Therapeutic Agents that this was a formidable task that required the knowledge, prestige, and talents of an organization like the National Academy of Sciences to undertake this comprehensive and vital task. The Academy of Sciences is particularly suited to handle this type of program and Dr. M. Robert Clarkson, Chairman of its Animal Health Committee, felt that this

*Department of Microbiology, New York State Veterinary College at Cornell University, Ithaca, New York.
undertaking was ideally suited for the objectives of that organization. To engender support of the veterinary community it was deemed worthwhile to canvass many organizations concerned with veterinary microbiology before the National Academy of Sciences was approached with an official request to undertake this project—these organizations were as follows: the newly formed American College of Veterinary Microbiologists; Poultry Disease Subcommittee of National Academy of Sciences; Biologics Committee of United States Livestock Sanitary Association; Committee on Animal Health of National Academy of Sciences; Conference of Veterinary Laboratory Diagnosticians; Veterinary Biological Licenses Committee of Animal Health Institute; and the Western Hemisphere Committee on Non-primate Animal Virus Characterization Committee of United States Livestock Sanitary Association. In addition certain key individuals in government organizations such as United States Department of Agriculture, National Institutes of Health, and Communicable Disease Center were consulted. Without exception all organizations and individuals agreed with the general plan and endorsed the idea of establishing in the National Academy of Sciences a committee that will have the responsibility to produce standardized methods in veterinary microbiology.

The National Academy of Sciences has agreed to undertake this project for which they are ideally constituted. Financial support was given recently by the Agricultural Research Service, USDA, to the Academy to support the activities of this committee. Dr. Charles J. York has agreed to serve as the chairman and nine other individuals were recently invited to become members of the committee. These individuals will represent various disciplines of veterinary microbiology. Although there are representatives from government, industry, and universities on the committee they were selected for their expertness in one of the disciplines essential to the successful production of standardized methods. As a member of a National Academy committee they represent no one except the Academy in the fulfillment of their charge and accordingly function without biased or vested interest. To bring this effort to this point we are indebted to many people but, in particular to Dr. M. Robert Clarkson (FDA), Dr. Robert Hanson (University of Wisconsin), Dr. John Hejl (USDA), Dr. L. Meyer Jones (AVMA), and Dr. Howard Sprague (NAS). There have been four planning meetings of Ad Hoc groups to date so we have made a reasonable beginning. The first meeting of the newly constituted Academy committee will be held this fall or early winter.

Now it is one thing to write standardized methods but it is another thing to utilize them to the best advantage. We want the manual(s) published by the committee to become as important to our profession as the handbook on Methods for the Examination of Poultry Biologics that was written by Poultry Disease Subcommittee on Animal Health of the National Academy of Sciences. Of necessity, the proposed manual(s) of the new Academy committee will be more comprehensive in subject matter than the poultry handbook and cover the other animal species of importance to the veterinary profession. This manual(s) should become a standard reference text(s) for all veterinary microbiologists interested in standardized
methods. To make this possible the manual(s) must be well planned and sensitive to the needs of our profession at large. To accomplish this objective the NAS committee will solicit ideas from all groups such as yours in an effort to do the best possible job.

To make the manual(s) more meaningful, veterinary microbiologists must designate reference microbiological strains to represent the various disease producing agents. This information will be included in the manual(s). In your organization the Non-primate Animal Virus Characterization committee now is designating tentative-working or reference strains of virus. In some instances only working strains could be designated until more knowledge becomes available for a given virus. This information is being catalogued and will become readily accessible to the scientific community. These reference strains are important in the production of a nomenclature of viruses. These designated reference or working strains will be readily available to scientists through the American Type Culture Collection which maintains a repository of such agents. Then we are in an excellent position to produce viral reagents which will give more uniformity and greater accuracy in the diagnosis and research of animal diseases.

The manual(s) should also include information that is written and organized in such a manner that it will be of great value and use to the veterinary laboratory diagnostician.

The manual would have unlimited value to individuals and organizations that are concerned with the production and control of veterinary biologics. Standards are vital to this phase of veterinary medicine. With the availability of standardized methods and excellent reagents veterinary biologics should improve and this will benefit the practitioner and the public he serves.

There are many by-products to a venture of this type in which many microbiologists and others interested in veterinary standards will contribute either directly or indirectly to the writing of the manual(s). As they are confronted with this complex and comprehensive task certain solutions to problems will be made and other probable solutions will be made manifest to the group. In the process new problems will be recognized and this will assist the researcher in his approach to many unsolved problems of practical importance to our profession in its attempts to control and prevent animal disease.

Until now we have not touched upon the use of veterinary biologics by the practitioner. It is the responsibility of individuals in regulatory veterinary medicine to see that the practitioner receives the best available current knowledge on the value and use of veterinary biologics. This is one of the objectives of the AVMA Council on Biological and Therapeutic Agents. Recognizing that a certain gap exists in this regard, the Council at its last meeting passed a motion that requested authorization to establish and administer a Board of Experts for the purpose of evaluating information on veterinary biologics as a means of guiding the veterinary profession in the proper use of such products. In addition, the Board must use every means of communication at its disposal to transmit its
recommendations to every veterinary practitioner in the United States. The Board would be composed of experts sufficient in number to represent the various disciplines concerned with veterinary biologics. Recognizing that its members would not have experts on all biologics under review, the Board should be able to invite other experts when required to serve as consultants on Ad Hoc basis. At its next meeting the Council will discuss the operation of the Board in detail with a member of the medical profession who serves on a Board which evaluates human biologics for human practitioners. It will then present its final recommendations to the officers of the AVMA for approval and implementation. We feel that this is an important link that will render an invaluable service to the veterinary practitioner, the biological producer, and the Veterinary Biologics Division of the ARS, USDA.

In conclusion I know that you will agree that the programs I discussed are essential to the future welfare and competency of our profession. The willingness of many organizations and individuals to participate actively in these programs that involve the use of standardized methods is strong evidence that the time is right to accomplish the objectives alluded to in this presentation. Our profession should be grateful to the foresight of many organizations, in particular, the AVMA, USLSA, USDA, and NAS for their active support of these programs. These programs won't be completed overnight—in fact all of these ventures will require the patient understanding and the energies of many individuals that must frequently communicate with each other over a long period of time.
THE ROLE OF THE STATE IN SURVEILLANCE OF VETERINARY BIOLOGICS

B. S. Pomeroy, D.V.M., M.S., Ph.D.* and John Newman, B.S., D.V.M.*

St. Paul, Minnesota

During the past two years renewed interest has occurred in the need to assure the veterinarian and livestock and poultry producers that veterinary biologics are free from contamination, pure, safe and potent. This renewed interest was sparked specifically by reports that certain poultry live virus vaccines were found contaminated with Mycoplasma gallisepticum and Newcastle disease virus. As a result of this recent episode it is apparent that state and federal agencies in cooperation with the biological industry have important roles to play in order to minimize a repetition of this problem.

As a result of this current situation the United States Department of Agriculture, the Federal Agency responsible for the administration of the Virus-Serum-Toxin Act of 1913, has reevaluated its program and requested additional financial support to increase its supervision of the production of veterinary biologics. It is the objective of this paper to look at the contributions the state agencies may make in supplementing the federal program.

In order to obtain some background material for this paper, a questionnaire was sent to each state official in charge of animal disease control programs.

The areas covered in the questionnaire were as follows:

1. Do you have a state licensing program for veterinary biologics produced in your state?
2. Does this program entail the inspection of the facilities and examination of the products for purity, safety and potency?
3. Do you have any biological producer in your state not under federal license and not supervised by a state agency?
4. Do you have regulations controlling the sale and distribution of veterinary biologics in your state?
5. Do you permit the production and use of live culture Mycoplasma gallisepticum for the controlled exposure program in chickens in your state?
6. Has your office ever had occasion to report to the Veterinary Biologics Division that a biological product manufactured under Federal License was not up to standards?
7. When a biological manufacturer was requested by Veterinary Biologics Division to remove from the market an unsatisfactory product, was there any effort made on the part of state agency to

*Department of Veterinary Bacteriology and Public Health, College of Veterinary Medicine, University of Minnesota.
see that all involved serial numbers were actually recalled from the market?

8. What suggestions do you have that state animal disease regulatory and research agencies may do to more effectively monitor veterinary biologics and supplement the program of the Veterinary Biologics Division.

The results of the survey reveal certain shortcomings at the state level. Replies were received from 37 of the 50 states.

1. Only eight of the 36 states reported having specific rules and regulations governing the licensing of the production and/or sale of veterinary biologics.

2. The programs varied in detail from only a licensing power to the power of inspection of the facilities and testing of the biologics for purity and potency as may be deemed necessary.

3. Thirty-six of the 37 states indicated that all producers of veterinary biologics in the respective states were under Federal or State Programs. In one state there was no supervision of the production of poultry biologics. In the states reporting there were very few manufacturers producing biologics for intrastate use.

4. Thirty-one of the 37 states have some type of regulations that control the sale and distribution of veterinary biologics produced under federal license. In some states the control is limited to products containing living organisms or only to products produced under limited or special federal license.

5. In 12 of the 37 states live virulent culture *Mycoplasma gallisepticum* is used under restricted basis for the controlled exposure of chickens. This immunizing agent cannot move interstate but it does indicate the widespread use of a product that is produced under no uniform procedure.

6. Nine of the 37 states have reported to the Veterinary Biologics Division over recent years that biological products manufactured under Federal License were not up to standard. All except one of the complaints involved poultry biologics.

7. When a biological product was requested to be withdrawn from the market by the Veterinary Biologics Division, only 15 of the 37 states made an effort to see that all involved lots were actually withdrawn from the market in their states.

Utilizing the information received from state veterinarians, veterinary diagnosticians and research workers, the following suggestions are made in order to assure that the highest quality of veterinary biologics are available.

1. At the Federal level the Veterinary Biologics Division must develop a monitoring program that will reasonably assure that all lots of veterinary biologics for interstate use are safe, pure and potent prior to their release for sale. This is a responsibility of the Federal government. It is financially impossible for the states
to adequately test biologics for safety, etc., and it is a needless, costly duplication of activities.

2. The United States Department of Agriculture publish in the Federal Register standard requirements for the detection of contamination in veterinary biologics and tests for safety, purity and potency of veterinary biologics. This information should be available to the public for review and constructive criticism.

3. The Veterinary Biologics Division develop an advisory committee that may serve as consultants in reviewing and establishing standards for veterinary biologics.

4. At the state level the animal disease control Agency should develop rules and regulations controlling the sale and distribution of all veterinary biologics.

5. The state Disease Control Agency (DCA) should encourage the reporting by veterinarians, diagnosticians and livestock and poultry producers, all complaints against veterinary biologics.

6. The state DCA should have available an epidemiologist to investigate complaints involving biologics. This individual should work very closely with the official diagnostic laboratory and research workers.

7. The state epidemiologist should communicate with the Veterinary Biologics Division as quickly as possible with regard to investigations concerning an unsatisfactory product.

8. When a veterinary biologic is recalled from the market because of contamination, etc., the Veterinary Biologics Division should notify at the state level: Disease Control Agencies, Official Diagnostic Laboratory, Dean or Head of Veterinary College or Veterinary Science Department of such action.

9. Federal and state agencies should coordinate their activities to make sure the recalled product is removed from the market.

10. Require adequate records be maintained by the manufacturer and their distributors of veterinary biologics so each product can be traced to its final user and when necessary be rapidly withdrawn from the market.

Investigations of Complaints

As more and more control and eradication programs develop for livestock and poultry, investigations of "breaks" in specific pathogen free herds and flocks and complaints of unusual reactions to biologics become paramount. The illustration that follows points out the importance of a state surveillance program involving the cooperative efforts of an epidemiologist, diagnostic and research laboratories, state and federal disease control agencies, and the livestock and poultry industries.

An outbreak of infectious sinusitis was reported by hatchery A involving a turkey breeding flock in June 1964. The disease appeared in other breeding flocks on two associate farms over the next two months. The involved flocks were marketed and the premises were thoroughly
cleaned and disinfected. Epidemiological studies failed to reveal a satisfactory explanation of the original source of the infection. About six months following the original "break" history repeated itself in the replacement flocks placed on these farms. Studies showed that the involved flocks had titers to Newcastle disease virus (NcD) as well as to Mycoplasma gallisepticum (MG). The serological tests at the time of selection of the flocks were negative to these diseases. After intensive repeated inquiries of the owner and employees by poultry epidemiologists of the Animal Health Division of the United States Department of Agriculture assigned to the project, it was decided to investigate the vaccines used on the flocks. The flocks were vaccinated at the time of selection or shortly afterwards with a live virus pox vaccine as well as with killed bacterins for fowl cholera and erysipelas. The investigation revealed that the same lots of fowl pox vaccine were involved in the outbreak in June, 1964 as well as the second one in January, 1965.

About the time the laboratory investigations with the fowl pox vaccine from hatchery A were underway, hatchery B reported an outbreak of IS in a breeder flock. Inquiry revealed the same brand of fowl pox vaccine was used and more intense investigations indicated two of the same serial numbers were involved. The disease patterns were similar. The flock became serologically positive to Newcastle disease as well as MG infection.

Intensive laboratory studies indicated the fowl pox vaccine was contaminated with Newcastle disease virus and with MG. A preliminary report was made to the Veterinary Biologics Division and the contamination of the vaccine with NcD virus was supported but tests for MG were inconclusive.

In the fall of 1965 hatchery C reported an outbreak of IS in a MG negative breeding flock. Other flocks subsequently came down with the disease. The same brand of fowl pox vaccine was involved but a different serial number. The same serological patterns were observed as in the previous flocks. Laboratory studies revealed the contamination of this serial number. These observations were reported to Veterinary Biologics Division and their studies supported the findings of MG contamination.

In conclusion, the state animal disease control agency has an obligation to the practicing veterinarian and the livestock and poultry producers to maintain a constant surveillance program on veterinary biologics. The practicing veterinarian has an important role to report to the proper authorities unusual incidents involving veterinary biologics. The livestock and poultry industries must give support on the state level for better rules and regulations governing the sale and distribution of veterinary biologics. The biological industry in cooperation with the Federal Government must constantly improve the standards of testing and production of veterinary biologics in order to assure that all lots of biologics are free of contamination, safe, pure and potent prior to their release for sale.
REPORT OF THE COMMITTEE ON BIOLOGICS

R. P. Hanson, Madison, Wisconsin, Chairman; N. Casselberry, Berkeley, California; J. M. Hejl, Hyattsville, Maryland; R. A. Heubner, Athens, Georgia; T. A. Ladson, Baltimore, Maryland; P. Langer, Kansas City, Missouri; J. C. Trace, Fort Dodge, Iowa; C. D. Van Houweling, Ames, Iowa; H. G. Wixom, Sacramento, California

Production and Testing of Biologics

The magnitude of production of biologics in the United States is clearly established by the figure of 1,421,767,886 cc produced during this last fiscal year by 55 licensed establishments (Table I). The diversity of the production is illustrated by the use of 142 categories to classify the products. The greatest of volume of production in any category is 179,468,649 cc of anti-hog-cholera serum. The greatest number of doses for any category is 2,779,052,000 doses of the combined Newcastle disease bronchitis vaccine; the volume of which 60,999,385 cc is also larger than that of any other vaccine.

The Veterinary Biologics Division of the United States Department of Agriculture, either at the National Animal Disease Laboratory in Ames or by other arrangements has tested selected serials for sterility and potency (Table II). Over 36 hundred serials from 58 of the 142 categories were examined. Two hundred and fifty-nine or seven percent were rejected because of the presence of contamination or lack of potency. The percent of serials rejected for a given category tested varied from none to as high as 33 percent. The percent sampled was as low as 3.8 percent and as high as 100 percent, the latter percent being examined when a high rejection rate was found on initial sampling. The serials rejected were either not released or if the manufacturer has already released them, they were recalled from the market.

TABLE I

Biological Products Produced in Licensed Establishments During Fiscal Year Ended June 30, 1966

<table>
<thead>
<tr>
<th>Classes</th>
<th>Product categories</th>
<th>cc</th>
<th>dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>vaccines and viruses</td>
<td>53</td>
<td>423,519,078</td>
<td>5,109,418,991</td>
</tr>
<tr>
<td>bacterins</td>
<td>36</td>
<td>615,517,556</td>
<td>211,611,543</td>
</tr>
<tr>
<td>serums</td>
<td>20</td>
<td>326,277,744</td>
<td>6,934,150</td>
</tr>
<tr>
<td>mixed bacterins</td>
<td>9</td>
<td>23,429,377</td>
<td>6,934,150</td>
</tr>
<tr>
<td>diagnostics</td>
<td>8</td>
<td>2,927,104</td>
<td>52,331,870</td>
</tr>
<tr>
<td>antitoxins</td>
<td>5</td>
<td>3,389,850</td>
<td>338,985</td>
</tr>
<tr>
<td>other biologics</td>
<td>11</td>
<td>26,702,177</td>
<td>7,549,230</td>
</tr>
<tr>
<td>Total products produced</td>
<td></td>
<td>1,421,762,886</td>
<td>5,412,963,885</td>
</tr>
<tr>
<td>Total products destroyed</td>
<td></td>
<td>42,019,190</td>
<td>273,732,101</td>
</tr>
<tr>
<td>Net production</td>
<td></td>
<td>1,379,743,696</td>
<td>5,139,231,784</td>
</tr>
</tbody>
</table>

75
TABLE I

Results of Testing of Biologics During Fiscal Year Ended June 30, 1966

<table>
<thead>
<tr>
<th>Categories tested</th>
<th>Serials produced of the categories tested</th>
<th>Serials tested</th>
<th>Percent tested</th>
<th>Serial rejected sterility</th>
<th>Serials rejected sterility</th>
<th>Percent rejected sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>9771</td>
<td>3687</td>
<td>37%</td>
<td>92</td>
<td>167</td>
<td>77%</td>
</tr>
</tbody>
</table>

Not all biologicals are produced under regular license. Fourteen products, several of them accounting for a significant volume of total production, are produced under a special license issued on a continuing basis. Eleven others are on special licenses with specified termination dates. Any of the licenses can be considered for reissue.

Five requests for field trials of veterinary biologics were denied. Three of these were for products produced abroad. In addition, two import permits for resale were denied. One product, porcine origin, modified live virus, hog cholera vaccine, was eliminated.

Contamination of vaccine serials, uncovered largely after release of the serial, has been a major problem. Largely unreported in Table II, because many of the observations have been made since June 30, is the detection of mycoplasma in 23 of 267 serials of poultry vaccines examined. In part this discovery can be attributed to improved methods for culturing mycoplasma. However, the fact that large numbers of organisms were found in some samples and an extraneous virus was recovered in other instances suggests that implant surveillance had been lax. Recall of the serials in question from the market did not come in time to prevent rather wide use. Litigation which has ensued and the publicity that it is receiving has seriously undermined confidence in poultry biologicals in a large segment of the poultry industry.

Current Research on Biologics

In addition to the random testing of serials of vaccines, the Veterinary Biologics Division at the National Animal Disease Laboratories carries on an active research development program. Most of the sixteen current projects are directed to the improvement of procedures for the safety or potency testing of biologicals. The kinds of subjects under study include suitability of culture media, the replicability of assay systems, the relationship between two or more testing procedures, the significance of one or more environmental variables and the comparison of strains. Vaccines and bacterins for poultry and for small and large animals are all covered in these investigations.

The Division is also collaborating with a European group that is seeking to develop international reference preparations for biologics. The initial trial will be an assay of an anti-Newcastle disease vaccine.

Research in progress in state and private institutions that can be related to improvement of veterinary biologics is not readily evaluated since it is based on unpublished work now largely in the form of progress
reports to funding agencies or on oral presentations and discussions at recent meetings. As such, it is fragmentary and subject only to preliminary categorization.

A large number of institutions are engaged in such research. Based on available notes, 35 institutions have investigations related either to the study of 10 agents now used in the production of biologicals or 11 agents or complexes for which the investigators believe biologicals may some day be produced. Twelve investigators have directed efforts to the improvement or the application of serological methods such as the fluorescent antibody test and gel precipitation test, and to new cultural systems such as the development of cell lines or to new methods for increasing sensitivity of cultural techniques. Four institutions are developing procedures that will improve the sensitivity of safety tests, six are concerned with potency test procedures and three with new systems for the production of biologicals. Three institutions reported studies on evaluation of vaccine serials of specific interest, and at least six institutions cooperated with the federal government in detecting contaminated serials of poultry vaccines.

**National Organizations and Meetings**

Three American organizations have sections devoted to improvement of veterinary biologics. The membership of the Veterinary Biological Licensees Committee of the American Health Institute consists of representatives of companies producing veterinary biologics. Their stated objectives are to improve the quality of biological products used in diagnosis, prevention and treatment of animal disease; to collaborate with appropriate biological regulatory agencies concerning mutual biological problems; and to promote the best interests of the livestock industry.

The National Academy of Science is an independent agency which has the tradition of serving the government as a scientific advisor. Its membership is made up of scientists from academic institutions, government agencies and industry. Two subcommittees of its Animal Health Committee are concerned with the evaluation of biologics. The Poultry subcommittee prepared and published *Methods for Examination of Poultry Biologics* and is now considering the third revision of that manual. The second subcommittee, newly organized, is concerned with the development of a manual for standard methods in Veterinary Microbiology. It is envisioned that this manual will eventually treat all veterinary biologics. These subcommittees were funded this year by a grant to the National Academy from the U.S. Department of Agriculture. The American Veterinary Medical Council on Biological and Therapeutic Agents was instrumental in the establishment of the new NAS subcommittee and in obtaining support for the Animal Health subcommittees.

Several organizations have held symposia in 1966 in which the production or evaluation of biologics was discussed. The American Veterinary Medical Association Council on Biological and Therapeutic Agents and the American Kennel Club sponsored a symposium on "Canine Distemper Immunization" in February. Papers were presented on the evaluation of vaccines and immunization procedures. The New York Academy of
Science organized a symposium on Mycoplasma that met in New York in May. Many facets of the biology of these organisms were presented. Also in May, the Communicable Disease Center and the American Veterinary Medical Association sponsored a symposium on rabies in Atlanta. Featured were the latest methods used in the control of rabies. The Communicable Disease Center and the Kansas State Veterinary College and the Kansas Medical Center presented a symposium in Manhattan this September on "Diseases Common to Man and Animals." Most of the papers presented in these symposia will be published in special publications.

The Importance of International Organizations in the Evaluation and Development of Biological Products

The activities of the FAO (Food and Agriculture Organization) working jointly with the WHO (World Health Organization) in the study of zoonoses, have and will continue to focus important attention on known and emerging diseases which will surely indicate the necessity for new and improved biological products to adequately cope with these diseases in the animal hosts.

The work of expert committees in these organizations will be valuable in the development of standards for and regulation of biological products throughout the world. Familiarity with the activities of this joint study and with the activities of the older organization; Pan American Health Organization (formerly Pan American Sanitary Bureau), will be of increasing importance in the development of standards for biological products in the United States. Our goal must be to make sure that the products available in the United States are as safe and effective as they must be and as good or better than any in the world.

The Pan American Health Organization is holding an international conference in Washington, D.C., November 7-11, 1966. The agenda of this conference is devoted to vaccines against viral and rickettsial diseases of the man. However, in the area of influenza virus vaccines, the problems of the animal reservoirs could be important from the standpoint of a possible need for more attention to animal immunization in the future. Another important consideration of this program is, "Problems and Future of Immunologic Adjuvants." This is of equal importance as applied to veterinary vaccines in view of the need for adjuvants in animal biologics to reduce the dosage frequency to a minimum to obtain a proper immune status and yet be able to avoid tissue residues in animals injected with adjuvant containing products. The agenda also will contain much of interest to any microbiologist in the human or veterinary field concerned with the evaluation and development of virus vaccine.

We feel all those concerned with the regulation, preparation or performance of biological products should be aware of the international activities in these organizations in order to keep our effort in these areas directed toward development of those biologics that will make their application acceptable in any country of the world to control animal diseases of both public health significance (zoonoses) or for prevention of animal diseases that endanger our world food supply.
THE CAPILLARY TUBE AGGLUTINATION TEST
IN THE CONTROL OF ANAPLASMOSIS

C. Joseph Welter, Ph.D.*

Des Moines, Iowa

INTRODUCTION

There are presently two tests which are used for serologic diagnosis
of anaplasmosis. The complement fixation (CF) test has been used for
nearly 12 years and has been reported to be a reliable test although it is
not a convenient test for extensive survey and control work. A more re-
cent test—the Capillary Tube Agglutination Test or CA Test was intro-
duced four years ago by Ristic.1 This test is conveniently used in ana-
plasmosis research programs and it has been designed in the form of a
kit (Anatest Kit, Diamond Laboratories, Inc., Des Moines, Iowa) for
routine use in the veterinarian’s office or diagnostic laboratory.

The accuracy and specificity of the CA Test was confirmed2,3 and
since then at least nine different laboratories have reported the test to be
an accurate and convenient method for detecting carriers of Anaplasmamarginale.4-13

Antigen used in the CA Test is derived from the erythrocytes of cat-
tle which show a high parasitemia with Anaplasma marginale. The antigen
is subjected to numerous purification steps in order to eliminate erythro-
cytic stromata and concentrate the initial and marginale bodies of A.
marginale.

The CA Test has been approved for use by the practicing veterinarian
in 20 states. In 16 states it is being used only in diagnostic laboratories.
Ten states have not granted permission to either diagnostic laboratories
or veterinary practitioners to use the CA Test. The CA Test is now rec-
nognized by the United States Livestock Sanitary Association as an official
test for the detection of A. marginale infection.

The CA Test has been very well accepted in many foreign countries
both as a routine research tool and as a method of determining disease
incidence where large numbers of serums require testing. Several sur-
veys on disease incidence have been recently completed. Under these
circumstances, the test will also detect carriers of A. centrale, but not at
the same degree of accuracy as A. marginale.

CONTROL METHODS FOR ANAPLASMOSIS

There are three possible methods for controlling anaplasmosis
whereby the CA Test can be effectively utilized:

*Research Department, Diamond Laboratories, Inc., Des Moines, Iowa.
1. Complete herd testing followed up by removal of carrier animals from the herd.
2. Elimination of carrier animals by tetracycline therapy.
3. Immunization.

Any efficient control program requires an accurate convenient test which will detect Anaplasma carriers. Complete herd testing utilizing the CA Test followed by removal of carrier animals has proven successful. In one study, a herd of 1000 cattle with a history of repeated outbreaks and losses due to anaplasmosis were tested. Seventy-two reactors were identified and removed from the herd. The test and segregation program proved to be highly successful in that losses were not reported during the subsequent vector season. On the other hand, herds in the same general area experienced outbreaks of anaplasmosis.

Another example whereby anaplasmosis has been controlled by application of the CA Test concerned an outbreak in Iowa in a herd of 85 cattle some of which had been shipped into Iowa from an anaplasmosis endemic area. Five deaths occurred and other cattle were visibly sick with anaplasmosis. At this time, all animals were tested with the CA Test. Twenty-three (32 percent) were CA positive and 57 were CA negative. The CF test was also performed on 54 of the 80 serums and data on both tests are compiled in Table I.

Percent agreement between the CA and CF tests (excluding 21 CF anticomplementary and suspect sera) was 91 percent. This is in agreement with data published by other laboratories. Two of the three cattle which were CA negative and CF positive were retested three months later and found to be strong CA reactors. These two animals were undoubtedly in the early stages of anaplasmosis and had not yet developed CA antibody. The third animal had been sent to slaughter along with CA reactors. Of the 21 CF suspect and anticomplementary serums, five were CA positive and 16 were CA negative. The CA Test is, therefore, more practical than the CF test since anticomplementarity does not affect the test and fewer suspect reactions are observed.

| TABLE I |
| Comparative CA and CF Serology on a Herd Experiencing an Outbreak of Anaplasmosis |

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>54</td>
<td>15</td>
<td>18</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>CA</td>
<td>54</td>
<td>17</td>
<td>37</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CF pos. in CA</td>
<td>15</td>
<td>12</td>
<td>3**</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CF neg. in CA</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CF susp. in CA</td>
<td>12</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CF AC in CA</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

*Pos. – Positive, Neg. – Negative, Susp. – Suspect, Ac. – Anticomplementary.
**Two animals tested later were strong CA positive, the third animal had been slaughtered.
At the end of the summer, all cattle were again CA tested and reactors were again removed from the herd. All cattle including replacement stock were retested a third time during the winter months and found to be negative. No additional outbreaks have been reported from this herd during subsequent vector seasons, thereby, indicating successful control of the disease by the CA Test and carrier removal program.

For more practical reasons, herds suspected of containing Anaplasma carriers should be CA tested two to four months after the vector season, thereby avoiding the necessity of multiple testing, in order to detect new carriers.

The success of this program may, of course, depend upon the type and incidence of vectors and their relationship to disease transmission. Control of anaplasmosis by removal of all carrier animals from a herd would be less effective in areas where ticks are the primary vectors. The primary vectors of anaplasmosis in the two herds previously described were tabanids.

Another method of controlling anaplasmosis—one that has received considerable attention during the past five years is the continuous oral administration of tetracycline for 30 or 60 days in order to eliminate the carrier status. This method of control is rather costly and cattle may not readily accept the drug for several days. In addition it may not be suitable under range conditions. This control program also requires the availability of a convenient test which will accurately identify the negative or carrier status of tetracycline treated cattle.

The CA Test is more accurate than the CF test in determining the negative or carrier status of treated animals. In a limited test two nine month old Anaplasma carrier steers—one splenectomized and the other intact—were injected intravenously with oxytetracycline (five mg. per lb. body weight) daily for 14 days. Both steers were strong CA and CF positive prior to treatment. Seven days after the last treatment (21 days after initial treatment) both steers became negative in the CA Test, but remained strong CF positives for an additional two weeks (Table II). At this time, 500 ml. of blood from each steer was transfused into one splenectomized calf each. Neither calf developed anaplasmosis nor CA or CF antibody during a two month observation period. Both treated steers were still CF suspects and CA negative two and one-half months after terminal treatment at which time both animals were inoculated with five ml. of blood from a virulent A. marginale carrier. Both animals developed acute anaplasmosis. These results indicate that both steers were completely freed of Anaplasma by injection of oxytetracycline. Serologic data indicates, however, that both animals, although CA negative, were still CF suspects two months after they were proven negative for Anaplasma by subinoculation into splenectomized calves. Furthermore, both steers were completely susceptible to anaplasmosis after elimination of the carrier status.

Similar observations regarding serology of treated animals also have been reported by Jatkar and associates. In these studies, three treated bulls were still CF positive three months after intravenous
TABLE II
Serologic Reactions of *Anaplasma marginale* Carriers Treated with Oxytetracycline

<table>
<thead>
<tr>
<th>Days</th>
<th>Spl. CA</th>
<th>Steer CF</th>
<th>Intact CA</th>
<th>Steer CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>14</td>
<td>3+</td>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3+</td>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3+</td>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td>Post-terminal Treatment</td>
<td>7</td>
<td>N</td>
<td>4+</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>15*</td>
<td>N</td>
<td>4+</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>N</td>
<td>2+</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>N</td>
<td>2+</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>N</td>
<td>2+</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>N</td>
<td>2+</td>
<td>N</td>
</tr>
</tbody>
</table>

*Five hundred ml. of blood transfused from each animal into one splenectomized calf each failed to transmit anaplasmosis.*

Treatment with oxytetracycline although negative in the CA Test. The negative status of these animals was confirmed by subinoculation of blood into a splenectomized calf.

In feeding trials at a consumption rate of five mg. chlortetracycline per pound body weight for 30 or 60 days, Franklin and co-workers reported positive CF titers in cattle for as long as five months after treatment. Only one of their test animals was tested in the CA reaction and it was negative. Blood from these cattle was not infective when inoculated into Anaplasma susceptible intact calves. In more recent studies by these workers, a comparison of the CA and CF tests on chlortetracycline treated cattle confirmed the advantages of the CA over the CF test. The greater accuracy and specificity of the CA test for testing treated cattle is probably due to the more highly purified antigen which is used in this test.

Eradication of anaplasmosis does not appear feasible in the United States particularly due to the residual infection in ticks, deer and other wild ruminants. Therefore, a third method of control requires consideration. This method is one of immunization.

The CA Test is an accurate indicator of the Anaplasma immune or carrier status of an animal. This is exemplified by the fact that cattle freed of Anaplasma by tetracycline therapy become CA negative and are again completely susceptible to anaplasmosis.

Inactivated vaccines prepared from either the purified CA antigen or unpurified CA antigen containing erythrocytic stromata, when combined with either complete or incomplete Freunds adjuvant, produced weak transient CA reactions in vaccinated cows. Strong CF titers were produced in cows vaccinated with the unpurified CA antigen. None of these cows resisted challenge with virulent *A. marginale* even after five injections (at four day intervals) of adjuvanted antigen. These observations
indicate the value of the CA Test in a control program utilizing immunization procedures.

SUMMARY

The Capillary Tube Agglutination Test has been shown to be a practical and essential part of any anaplasmosis control program. Its use in conjunction with three control programs has been demonstrated:

1. Complete herd testing followed up by removal of carrier animals from the herd.
2. Elimination of carrier animals by tetracycline therapy.
3. Immunization.

Under certain circumstances (following tetracycline therapy) the CA Test is more accurate than the CF test in determining the absence of Anaplasma infections. The CA Test is more convenient than the CF test for disease incidence studies where large numbers of serums need to be tested.

REFERENCES


FEEDING CHLORTETRACYCLINE TO RANGE CATTLE TO ELIMINATE THE CARRIER STATE OF ANAPLASMOSIS


There are several published reports on feeding high levels of chlortetracycline\(^t\) to eliminate the carrier state of anaplasmosis.\(^4,5,7\) However, there is little information available on the use of low level antibiotic feeding to eliminate anaplasmosis from reactor cattle. In 1958, Oklahoma researchers reported that daily group feeding of chlortetracycline at a level of 1.5 mg/lb/bw (mg/lb) for sixty days eliminated the carrier state in four experimentally infected cattle.\(^4\) Unpublished research at Texas A&M in 1961 demonstrated the effectiveness of daily hand feeding 1 mg/lb aureomycin for sixty days to eliminate anaplasmosis from a recently infected cow.

Franklin and co-workers in 1964 fed approximately 300 head of anaplasmosis infected range cattle 0.5 mg/lb of chlortetracycline daily for 120 days during the winter and spring.\(^3\) On the initial test 60 percent of the herd reacted to the complement-fixation (CF) test. Eleven months after the initial test and 120 days after antibiotic feeding, 92 percent of the herd tested negative to this test. One hundred percent were negative to the capillary agglutination (CA) test.

This paper is a report on the use of chlortetracycline fed daily at various levels (0.5 mg/lb to 5 mg/lb) and for various periods (30-120 days) to range cattle during the winter and early spring feeding period. Also a comparison between the CF and CA tests before and after antibiotic feeding is reported.

MATERIALS AND METHODS

Herd No. 1 consisted of 300 head of range cattle which were mostly Herefords but included some Angus. Sixty percent of these cattle reacted to the CF test prior to antibiotic feeding (treatment). Chlortetracycline was incorporated in protein (range) cubes to supply 0.5 mg/lb (0.5 mg daily per 1000 lbs. body weight) of aureomycin in two pounds of cubes per animal for daily consumption for 120 days. Blood samples for testing were collected prior to feeding antibiotic and 120 days after termination of feeding (Table I).

Herd No. 2 consisted of 300 head of range cattle predominantly

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\(^t\)Aureomycin\(^R\) is a trade name of the American Cyanamid Co., Princeton, New Jersey for chlortetracycline.
Herefords but including some Angus. This herd was divided into five groups of sixty animals each in separate pastures. The reaction to the CF test was from 70 to 75 percent. Each group was fed a certain level of chlortetracycline in range cubes daily as follows:

Group 1 was fed at a 1 mg/lb level (1 gm per head) for 30 days
Group 2 was fed at a 1 mb/lb level (1 gm/head) for 60 days
Group 3 was fed at a 2 1/2 mg/lb level (2 1/2 gms/head) for 30 days
Group 4 was fed at a 2 1/2 mg/lb level (2 1/2 gms/head) for 60 days
Group 5 was fed at a 5 mg/lb level (5 gms/head) for 30 days.
Blood samples for testing were collected prior to feeding antibiotic; at sixty days and at eight and nine months following the termination of feeding (Tables II and III).

Standard methods and procedures of collecting blood samples were used. Sera were tested by the CF and CA test procedures according to the official manual and pamphlet as published by the Animal Health Division, United States Department of Agriculture and Diamond Laboratories, respectively.\textsuperscript{1,2}

RESULTS

Group 1

The animals in Group 1 which were fed one gm of aureomycin for 30 days were tested 60 days after completion of treatment. Of this group, 32 head were positive to the CF test (CF positive); of these, 22 head (69 percent) were also positive to the CA test. Ten animals of the 32 (31 percent) were positive on the CF test, but negative on the CA test (CA negative).

Nineteen head (31 percent) of Group 1 were tested nine months after treatment. Thirteen animals (68 percent) were CF positive and CA negative; whereas, six animals (32 percent) were negative to both CF and CA tests. One hundred percent (100 percent) of 19 tested were negative to the CA test.

Group 2

The animals in Group 2 which received one gm of aureomycin for 60 days were tested 60 days after completion of treatment. Of this group, 42 head were CF positive; of these 35 (83 percent) were CA negative. Seven head (17 percent) of the 42 were positive to both CF and CA tests.

Twenty-six head (47 percent) of Group 2 were tested eight months after treatment; three head (12 percent) were CF positive and CA negative; three head were CF suspicious and CA negative, whereas 20 head (76 percent) were negative to both CF and CA tests. One hundred percent (100 percent) of 26 tested were negative to the CA test.

Group 3

The animals in Group 3 which were fed 2 1/2 gms of aureomycin for 30 days were tested 60 days after treatment. Of this group, 31 head were CF positive; of these 22 head (74 percent) were CA negative. Nine head (26 percent) of the 31 were positive to both CF and CA tests.

Forty-five head (70 percent) of Group 3 were tested nine months after treatment. Two animals (four percent) were CF positive and CA negative; one animal (two percent) was positive to both CF and CA tests, whereas, 42 head (94 percent) were negative to both CF and CA tests. Ninety-eight percent (98 percent) of 45 tested were negative to the CA test.
Group 4

The animals in Group 4 which were fed 2 1/2 gms of aureomycin for 60 days were tested 60 days after treatment. Of this group 38 head were CF positive; of these 31 head (82 percent) were CA negative. Seven head (18 percent) of the 38 were positive to both CF and CA tests.

Thirty-six head (64 percent) of Group 4 were tested eight months after treatment. One animal (three percent) was positive to both the CF and CA test. Thirty-five head (97 percent) were negative to both the CF and CA tests. Ninety-seven percent (97 percent) of 36 tested were negative to the CA test.

Group 5

The animals in Group 5 which were fed five gms of aureomycin for 30 days were only tested at 60 days after treatment. Of this group, 19 head were CF positive; of these, 17 head (90 percent) were CA negative. Two head (10 percent) of the 19 were positive to both CF and CA tests. Ninety percent (90 percent) of 19 CF positives were negative to the CA test.

DISCUSSION

After the encouraging results obtained from feeding a low level of chlortetracycline to 300 head in Herd No. 1, it was decided to feed various levels of aureomycin in a second herd and note any differences between the CF and CA test. It appears from the results in Herd No. 2, that a low level (1 mg/lb) 60 day feeding period is preferable to a 30 day period. The 2 1/2 mg/lb levels for 60 days appear to be more efficient than the same levels for 30 days in eliminating the carrier stage when tested 60 days after treatment. However, the results are comparable when tested eight to nine months after treatment.

It is to be noted that as the level of antibiotic or as the time of treatment is increased, non-agreement between the CF and CA tests is greater when tested at 60 days following treatment. However, with exception of Group 1, at eight to nine months following treatment, the CF and CA are in fair to close negative agreement (76 to 97 percent). The CA test in all groups tested at eight to nine months was 97 to 100 percent negative.

The persistence of the CF reaction in cattle following treatment with high level chlortetracycline and their failure to transmit anaplasmosis when inoculated into test calves has been reported. In a publication now in press, the authors have shown the efficiency of low and medium level feeding of chlortetracycline to eliminate the carrier stage under controlled conditions in intact and splenectomized calves. This would complement the test results of the two herds reported in this paper.

The authors advocate increased use of the CA test by regulatory agencies to determine the test's effectiveness under field conditions to eliminate reactors and help control the spread of anaplasmosis. Long term studies in well managed herds would be especially desirable.
Feeding Chlortetracycline to Range Cattle

Long range studies are continuing with Herd No. 2 which is in an area infested with the winter tick, Dermacentor albipictus. This type of area presents a continuing problem in preventing cattle from becoming reinfected.

In addition a group of young carrier cows will be the basis for long term study of low level feeding.

It is hoped that this paper will stimulate other studies on the practicality of low level antibiotic feeding to eliminate the carrier state of anaplasmosis.

Summary

Two range herds consisting of approximately 600 head of cattle were fed various levels of chlortetracycline. One herd of approximately 300 head were fed 0.5 mg/lb (0.5 gm per head) daily for 120 days during the normal winter feeding period. The initial herd CF reaction was 60 percent. One hundred twenty days after antibiotic feeding, the CF reaction was eight percent whereas, the CA test reaction was zero percent.

The other herd was divided into five equal groups and each group was fed separate levels of antibiotic: 1 mg/lb, 2 1/2 mg/lb and 5 mg/lb for 30 or 60 day periods. The initial herd CF positive reaction was 70 to 75 percent. Only in Group 1 which was fed one gm per head daily for 30 days was there fair positive agreement (69 percent) between the CF and CA tests at 60 days following treatment. Nine months after treatment, there was no positive agreement (all CA's were negative), but six head (32 percent) were in negative agreement (CF and CA negative).

As the length of time of treatment was increased from 30 to 60 days or as the treatment level was raised from one gm to 2 1/2 gms per head, non-agreement between the CF and CA reaction became much greater at 60 days following treatment. At eight or nine months after treatment, the CF and CA tests were in fair to close agreement (76 percent to 97 percent).

The authors advocate increased use of the CA test in the field by regulatory agencies to determine its value in the control of anaplasmosis.

References

1. Anon: Anatest, Diamond Laboratories, Des Moines, Iowa.

REPORT OF THE COMMITTEE ON ANAPLASMOSIS

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The beef cattle industry is very much concerned about anaplasmosis in the United States. Because of this increasing concern with a serious threat to economic cattle production in many areas, it was the objective of the Anaplasmosis Committee to develop a comprehensive report as possible. To accomplish this, members of the Anaplasmosis Committee were assigned specific areas of review and study on May 20, 1966. All five Sub-committees completed their assigned work prior to the meeting in Buffalo, New York.

The report, therefore, is the result of five months of thinking and work by members of each Sub-committee, whose reports were submitted to the entire Anaplasmosis Committee for review, revision and adoption in Buffalo, New York on October 10 and 11, 1966.

PROPERTIES OF THE CAUSATIVE AGENT

The causative agent *Anaplasma marginale*, which occurs as a marginal body in erythrocytes of cattle with anaplasmosis, was considered for many years to be a single parasitic unit. During the last decade, however, it was revealed by means of electron microscopy that the marginal anaplasma body is actually an inclusion body which usually consists of from two to eight smaller units. These smaller units, called initial bodies, have morphologic characteristics similar to those of rickettsiae and bacteria.1,2

The invasive mechanism of the initial Anaplasma body was found to involve penetration of the erythrocytic membrane by a yet undetermined mechanism. It was also found that in invaded erythrocytes, the initial Anaplasma body reproduces by binary fission.3 Like rickettsiae, Anaplasma organisms systematically invade tick tissues and multiply in them by binary fission.4

Anaplasma parasites, unlike Plasmodium and Babesia, do not deoxygenate hemoglobin.5 This finding is consistent with an earlier observation that the respiration rate of Anaplasma is far below that of typical blood protozoan parasites and approximates the respiration rate of rickettsiae and bacteria.6 Unlike typical blood protozoan parasites, Anaplasma
are susceptible to destruction by broad spectrum antibiotics. Recent evidence was obtained of accelerated glycine uptake into protein by Anaplasma-infected erythrocytes which suggests that while within its host cell, the organism might be effectively studied by capitalizing on the limited metabolic capabilities possessed by the mature mammalian erythrocyte.

References to long tail-like appendages attached to some of the marginal Anaplasma bodies have been reported to occur in certain isolates of Anaplasma and have been illustrated by phase and fluorescent microscopy. Basic immunoserologic and host specificity studies of the tailed parasite revealed that it is in part antigenically distinct from *A. marginale* and will not survive in deer hosts. The tailed parasite has been called *Paramplasma caudata*. On the basis of electron microscopic studies, it was concluded that the band-like structure is not an integral component of the parasite, but rather it is a result of the unusual organization of proteinoaceous erythrocytic material apparently caused by parasitism. Serologic specificity of the band-like structure as revealed by the fluorescent antibody technique indicates that it contains, at least in part, an antigen derived from the parasite.

**DESCRIPTION OF CLINICAL FORMS OF THE DISEASE**

There are mild, chronic, acute and peracute forms of anaplasmosis grouped according to the variation in severity and duration of the disease. The malady is generally mild in calves up to one year of age; acute but rarely fatal in cattle up to two years of age; acute and occasionally fatal in cattle up to three years of age; and often peracute and frequently fatal in cattle over three years of age.

Naturally occurring mild cases of anaplasmosis in calves are often asymptomatic; however, one may occasionally observe temporary depression, loss of appetite, dehydration, and mucopurulent, lacrimal, and nasal discharges in these animals.

Chronic anaplasmosis is manifested by slow recovery from an acute attack which persists from two weeks to three months, during which period anemia, icterus, anorexia, emaciation, and reduced milk production are observed.

The signs of acute anaplasmosis usually consist of anemia, weakness, febrile reaction, constipation, icterus, inappetence, depression, dehydration, labored respiration, and abortion. The acute crisis often occurs unexpectedly, with no prior evidence of illness.

Peracute anaplasmosis constitutes the most severe, usually fatal, form of the disease. It occurs frequently in purebread animals or in high-producing milk cows, which succumb within a few hours following the onset of infection. In addition to anemia, milk flow is suspended, extensive salivation and very rapid respiration are noted and animals so affected often exhibit irrational behavior and signs of nervousness.
EVALUATION OF CURRENTLY AVAILABLE SEROLOGIC TESTS

ANAPLASMOSIS

The complement fixation (CF) and the capillary tube agglutination (CA) tests are presently used for the serologic diagnosis of anaplasmosis. Antigens used in these two tests are derived from erythrocytes of cattle acutely infected with *A. marginale*.

The CF test has been in existence for nearly 12 years and has been found to be a relatively accurate tool for detecting Anaplasma carriers. The CA test was developed in 1962 and since then, it has been studied by numerous investigators and also found to be relatively accurate and a simple means for detecting Anaplasma carriers. In addition, several comparative studies of the CF and CA tests have been made on sera of anaplasmosis suspect cattle and these reports indicate that results obtained by these two tests agree within 92 percent and 100 percent.

The application of CF and CA tests to examination of sera from wild ruminants such as elk, antelope, deer, big horn sheep, etc., revealed that the CF test results are misleading and unreliable in these cases while the CA test was 97 percent accurate when applied to Anaplasma negative sera of the big game animals, as determined by subinoculation of splenectomized calves.

The specificity of the CA test antigen with reference to the diagnosis of bovine anaplasmosis was shown by the lack of cross-reactions with antisera against 14 highly prevalent pathogens of cattle. False CF positive reactions were observed in five cows with eperythrozoonosis. This was confirmed by subinoculation of the blood from each of these cows into five susceptible splenectomized calves.

The CA test is presently being used to detect and control anaplasmosis on four continents of the world.

It is concluded that the diagnostic accuracy of the CA test compares favorably with that of the CF test when the latter test is performed under optimal conditions. However, there are certain difficulties intrinsic to the CF test which do not make the test applicable under all conditions. For example, of 1,612 samples tested by CF, 112 samples were anticomplementary which means that these samples could not be evaluated by the test. Hemolized serum samples, which occur most frequently in hot weather, also cannot be accurately tested with CF.

An additional point of interest is that the CA test appears to be more efficient than the CF test when used to ascertain whether continuous treatment with tetracycline drugs has been effective in freeing cattle of Anaplasma. At least two investigators have established that the CA test depicts more promptly and more clearly which animals have been freed of Anaplasma.

EVALUATION OF VACCINES

1. Commercial Vaccine: In 1965, a killed Anaplasma vaccine called "Anaplaz" was introduced commercially. Reports indicating the efficacy and lack of efficacy have been made.
2. Experimental Vaccines: Apart from the above experiments, long-term studies on immunity in anaplasmosis were also conducted. In these studies, vaccine preparations consisted of whole blood, washed erythrocytes, the complement-fixing Anaplasma antigen, the Anaplasma antigen used in the CA test, the soluble erythrocytic antigen known as "proteamine sulfate (PS) antigen" and the free-serum antigen known as "exo-antigen." The infectious Anaplasma organisms contained in certain vaccine preparations were destroyed by various treatment such as 0.2 percent phenol, 0.1 percent phenol, 0.1 percent formalin, 1:20,000 merthiolate, or simply by lyophilization. These workers have been unable to induce clinically useful immune responses in susceptible mature cattle with any of these vaccines. The only difference noted between non-vaccinated control animals and animals vaccinated with certain killed Anaplasma preparations following their challenge with one to five ml. of carrier blood, was that vaccinated animals showed a parasitemia ranging within 60 percent to 80 percent of that present in non-vaccinated animals.

Inoculation of susceptible cattle with most of these vaccines, however, resulted in the appearance of agglutinating, precipitating and complement-fixing antibodies in their serum. From these studies, it has been concluded that a serum antibody per se is not instrumental in controlling the disease process in anaplasmosis. Thus, the presence of serologically demonstrable antibody is not indicative of acquired immunity.

The investigators who have failed by means of killed Anaplasma vaccine to produce clinically useful sterile immunity in susceptible cattle offered the following explanation concerning the mechanism of immunity in anaplasmosis. These investigators found that anaplasmosis carriers in which the infection was destroyed by chemotherapy did not retain adequate residual sterile immunity to enable them to withstand clinical re-infection with Anaplasma.

TICK VECTORS

Transmission of anaplasmosis over the world has been demonstrated under controlled conditions with at least 20 species of ticks, nine of which occur or have occurred in the United States. The fact that transmission can be effected by a certain tick under experimental conditions does not necessarily mean that the tick is a vector in nature. Great variations in biology, habits, host predilection and geographic distribution of ticks in their natural environment undoubtedly produce great variations in their roles as vectors of anaplasmosis.

At present, only two species of ticks now occurring in the United States appear to have sufficient experimental and epidemiological support to be considered important natural vectors of anaplasmosis (Dermacentor andersoni, D. occidentalis). Certain species capable of transmitting infection under experimental conditions may be eliminated from consideration as significant natural vectors (Argas persicus, Rhipicephalus sanguineus, Boophilus microplus), while others remain under suspicion as
vectors pending further investigation (*D. variabilis*, *D. albipictus*, *Ixodes scapularis*).

*Argas persicus* (fowl tick): Transstadial transmission has been demonstrated experimentally. Since cattle are only rare, accidental hosts, this tick is not important as a vector of anaplasmosis.

*Boophilus annulatus* (cattle tick): Transovarian transmission has been demonstrated experimentally. While once an important vector of anaplasmosis in southeastern United States, this tick is no longer a factor in anaplasmosis transmission in this country with its eradication in the campaign against bovine babesiosis.

*Boophilus microplus* (tropical cattle tick): Transovarian transmission has been demonstrated experimentally. In the United States, this tick is confined to limited areas in Florida and along the Texas-Mexico border. While a potential vector of anaplasmosis, it is not at present a significant factor in transmission in this country because of constant surveillance and control.

*Dermacentor albipictus* (winter tick): Transstadial transmission has been demonstrated experimentally, but efforts to demonstrate transovarian transmission have failed. The actual role of this tick as a vector of anaplasmosis is unknown. The further role of this tick as a vector of importance should be investigated.

*Dermacentor andersoni* (syn: *D. venustus*; Rocky Mountain wood tick): Transstadial and transovarian transmission have been demonstrated experimentally. Transmission was also accomplished with ticks removed from cattle with clinical anaplasmosis. This tick is very prevalent throughout rangeland areas of Oregon, Idaho, Montana, Wyoming, Colorado, Utah, Nevada and northern and eastern California where anaplasmosis is enzootic in the cattle population. The prevalence, distribution and host relationships of *D. andersoni* strongly suggest that it is an important natural vector of anaplasmosis.

*Dermacentor occidentalis* (Pacific Coast tick): Transstadial and transovarian transmission have been demonstrated experimentally. Transmission has also been demonstrated with ticks collected from cattle and deer in the natural environment and allowed to feed on susceptible cattle. This tick is considered the most important vector in hill and mountain rangeland areas of California, where its distribution coincides with that of major anaplasmosis areas and large deer populations known to harbor latent infections.

*Dermacentor variabilis* (American dog tick): Transstadial transmission has been demonstrated experimentally, but efforts to demonstrate transovarian transmission have failed. This tick is widely distributed in the United States, being especially prevalent in the eastern two-thirds. The role of *D. variabilis* as a natural vector of anaplasmosis will remain uncertain until it is shown whether or not generation to generation transfer of the agent occurs.

*Ixodes scapularis* (black-legged tick): Transstadial transmission has been demonstrated experimentally. This tick occurs primarily in the southeastern and southern states. While its actual role as a natural vector
is unknown in this country, it must be considered a potential vector on the basis of its similarity to *I. ricinus*, which in Europe is a proved vector of anaplasmosis capable of generation to generation transmission of *Anaplasma*.67,68

*Rhipicephalus sanguineus* (brown dog tick): Transstadial transmission has been demonstrated experimentally.69 This tick is widely distributed throughout the United States, but prefers the dog as host and is only rarely found on cattle. It cannot be considered a significant vector of anaplasmosis.

**INSECT VECTORS**

Experimental and epidemiological evidence points to horseflies as the most significant insect vector of anaplasmosis. In the United States, at least nine species of *Tabanus* have been shown capable of transmitting infection under experimental conditions: *T. abactor*,70 *T. americanus*,70 *T. atratus*,71 *T. equalis*,70 *T. erythraeus*,70 *T. fumipennis*,65 *T. oklahomensis*,70 *T. sulcifrons*,70,72 and *T. venustus*.70

Transmission by horseflies is effected only by mechanical means, or the direct transfer of blood from infected to susceptible cattle. To effect transmission of infection, this transfer must take place within a few minutes after the fly feeds on an infected animal, or during the short period fresh blood remains on the mouth parts.73 The most efficient horsefly vectors of anaplasmosis, therefore, are species such as *T. abactor* and *T. sulcifrons* which attack again immediately after their feeding is interrupted.73 Transmission by horseflies is proportional to the proximity of infected and susceptible cattle and the numbers of flies, and is favored by the presence of cattle with clinical anaplasmosis.66,73

Other flies such as stableflies (*Stomoxys*), deerflies (*Chrysops*) and hornflies (*Siphona*) are potential vectors of anaplasmosis on experimental or epidemiological grounds,73 but their actual importance as natural vectors is unknown.

Mechanical transmission has been demonstrated experimentally with mosquitoes of the genus *Psorophora*.74 Transmission was accomplished when approximately 1,500 *P. ciliata* and *P. columbiae* were transferred to a susceptible animal immediately after partially engorging on an animal with clinical anaplasmosis. Mosquitoes are not considered nearly as significant as horseflies as vectors of anaplasmosis. However, mosquito transmission must be considered when susceptible and infected cattle are concentrated close together and large numbers of mosquitoes are present.

**RECENT STUDIES ON VECTORS**

In enzootic anaplasmosis areas in the southern states, there is increasing evidence that blood-feeding insects, particularly horseflies, are important vectors of anaplasmosis. Close correlation has been noted between horsefly populations and the occurrence of clinical anaplasmosis and the development of complement-fixation titers indicating the acquiring
ANAPLASMOSIS

of the carrier state. Measures aimed at insect control were effective in reducing the incidence of clinical anaplasmosis and percentage of cattle acquiring the carrier state as shown by the complement-fixation test. It was shown by means of the complement-fixation test that calves became positive as soon as 30 days after being placed in herds of carrier cattle during the period of greatest horsefly population. It was demonstrated by the complement-fixation test that calves placed among carriers with low titers became infected as readily as those among cattle with high titers.

In California, further evidence that D. occidentalis is an efficient vector was obtained by demonstration of infection in ticks collected from cattle on a ranch in an enzootic area, anaplasmosis resulting when the ticks were allowed to resume feeding on a susceptible heifer. Work in Maryland with progeny of D. andersoni obtained from Montana and Wyoming failed to confirm the transovarian transfer of the agent reported earlier. However, recognition was made of the fact that the failure to demonstrate hereditary transmission might be due to differences between the laboratory conditions under which the trials were conducted and the natural habitat of the tick. Experimental trials with male D. andersoni ticks have shown that the agent of anaplasmosis can survive for a number of months, suggesting that male ticks of this specie in the natural state may be very efficient vectors of the disease.

Fluorescent antibody studies of nymphs of D. andersoni that fed on a calf with clinical anaplasmosis revealed bodies in the cytoplasm of cells of the malpighian tubes identical in size and morphology to marginal and initial bodies seen in infected bovine red blood cells. Arrangements of fluorescent bodies in pairs and groups suggested the probability of developmental forms of A. marginale. These bodies were observed in nymphs up to five days after detachment but not after 37 to 58 days, and they were never seen in control ticks. This preliminary work indicates the need for further intensive work aimed at clarifying the tick phase of the life history of A. marginale.

WILDLIFE RESERVOIRS AND THEIR EFFECT ON THE PROBLEM OF ANAPLASMOSIS CONTROL

Several types of antelope in Africa (duiker, blesbok, black wildebeest) and deer in the United States (Columbian black-tailed deer, mule deer, white-tailed deer) have been shown to be susceptible to A. marginale infection of bovine origin or to harbor the infection naturally, usually in latent form. Other wild ruminants in this country such as buffalo, elk and antelope might be susceptible to infection of bovine origin. Attempts to establish infection in numerous non-ruminant domestic, wild and laboratory animals have failed.

In California, a high incidence of latent infection has been demonstrated in deer, which occur in large numbers on rangelands used by cattle and are attacked by the same types of arthropod vectors of
anaplasmosis.\textsuperscript{84,85} The presence of an efficient vector tick, \textit{D. occidentalis}, which attacks both cattle and deer, plays an important role in maintaining and spreading Anaplasma infection.\textsuperscript{61,62} This Anaplasma-deer-cattle-tick relationship may be responsible for the high rate of exposure of cattle to anaplasmosis at an early age and the high incidence of carriers of infection in mature cattle. Similar relationships probably exist in other western states where anaplasmosis is enzootic.

The presence of wild reservoirs of Anaplasma infection in areas used for the grazing of cattle may increase the difficulty of controlling anaplasmosis through eradication. Studies in California have suggested that the infection may persist in these areas in the absence of bovine hosts because of the presence of deer carrying latent infection and ticks capable of transmitting infection from deer.\textsuperscript{84}

CHEMOTHERAPY OF ANAPLASMOSIS

A review of the literature on chemotherapy has been made by this committee and references are listed.\textsuperscript{88,89}

It is the opinion of this committee that the only compounds which have been shown to have anaplasmastatic properties are the tetracyclines and a recently reported group of chemicals known as dithiosemicarbazones. Twenty-two compounds of the alpha-dithiosemicarbazones were reported to be active against \textit{Anaplasma marginale} in the splenectomized calf.\textsuperscript{90}

This group of compounds is under investigation both in this country (United States Department of Agriculture) and abroad (Welcome Research Laboratories) and detailed reports should be available shortly.

Continuing investigation at Texas A&M has further confirmed that low level (0.5-1 mg/lb/bw) feeding of chlortetracycline* (CTC) is effective in eliminating the carrier state of anaplasmosis. Feeding different experimentally infected calves 2.5, 1, and 0.5 mg/lb/bw CTC, daily for periods of 45, 41, and 90 days respectively eliminated carrier infection.\textsuperscript{91}

As a routine feeding practice to eliminate the carrier state, this antibiotic is recommended to be fed during the regular winter time feeding period when anaplasmosis is commonly in a quiescent state. The antibiotic may be incorporated in pelleted or ground feed mixtures at the recommended levels.

Low level feeding of CTC promises to be a fairly economical method for eliminating anaplasmosis from infected herds. In many areas of the United States this could be a practical method of controlling this important disease and can be put into practice almost immediately.

Previous recommendations concerning the use of high level tetracyclines for treatment and elimination of the carrier are to be found in the United States Livestock Sanitary Association Proceedings for 1962 and 1965.

\*Aureomycin is the registered name of a chlortetracycline product of the American Cyanamid Co. of Princeton, New Jersey.
The following recommendations are added to those already in effect:

1. Treatment: For the purpose of destroying the carrier state of anaplasmosis the treatment shall consist of: the administration of one (1) mg. per pound of body weight of chlortetracycline orally each day for sixty (60) days or

2. The administration of 0.5 mg per pound of body weight of chlortetracycline orally for ninety (90) days.

3. Animals treated by either method, and to be considered as non-reactors, are to remain negative for two (2) consecutive capillary agglutination (CA) tests at an interval of 30 days following treatment.

4. Although we are in a relatively good position regarding anaplasmosis therapy, there is still considerable room for improvement. Therapy requiring 60-90 day feeding periods or 10-16 days of daily intramuscular or intravenous injections to eliminate the carrier state are tedious, time-consuming and expensive. A product that will eliminate the organism with fewer doses (preferably one) over a shorter time is needed. Destruction of infectivity of a carrier animal's blood without eliminating its immune status is also a goal worthy of achievement. The committee recommends continued search for therapeutically effective compounds. The discovery of a second group of effective agents (the dithiosemicarbazones) referred to above is a very important step in this direction as it encourages further research in this area.

CURRENT RESEARCH PROJECTS IN THE UNITED STATES

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Institution</th>
<th>Source of Funds</th>
<th>Personnel</th>
</tr>
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</table>

1. Anaplasmosis in Cattle

   Department of Veterinary Science, Louisiana State University, Baton Rouge, Louisiana

   National Institutes of Health

   George Dimopoullos

2. Biochemical, Immunologic, Radiologic and Pathologic Applications to the Study of Anaplasmosis and Eperythrozoonosis

   Department of Veterinary Science, Louisiana State University, Baton Rouge, Louisiana

   State

   George Dimopoulous
<table>
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<tr>
<td>3. Diseases of Cattle - Anaplasmosis</td>
<td>Department of Veterinary Science, Louisiana State University, Baton Rouge, Louisiana</td>
<td>Hatch-230</td>
<td>Lon E. Foote, Ava D. Odom, W. T. Oglesby</td>
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<td></td>
<td>Anaplasmosis in Cattle</td>
<td>Hatch-393</td>
<td>T. E. Franklin, Kenneth Kuttler</td>
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<tr>
<td>Control of Anaplasmosis</td>
<td>Wyoming State Veterinary Laboratory, Laramie, Wyoming</td>
<td>State</td>
<td>J. F. Ryff, G. M. Thomas, H. A. Hancock, R. I. Port</td>
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<tr>
<td>1. Immunologic Mechanisms of Blood and Vascular Disorders</td>
<td>Department of Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana, Illinois</td>
<td>National Institutes of Health</td>
<td>M. Ristic, K. Sibinovic, W. Schroeder</td>
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<tr>
<td>2. Nature and Significance of Soluble Blood Parasite Antigens Occurring Free in Serum</td>
<td>Department of Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana, Illinois</td>
<td>National Institutes of Health</td>
<td>M. Ristic, D. H. Ferris</td>
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<td>The Epizootiology of Bovine Anaplasmosis</td>
<td>School of Veterinary Medicine, Agricultural Experiment Station, University of California, Davis, California</td>
<td>Hatch</td>
<td>J. F. Christensen, J. W. Osebold, J. A. Howarth, J. R. Douglas, N. F. Baker, J. E. Moulton, R. B. Bushnell</td>
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<tr>
<td>Immunization Methods Against Anaplasmosis</td>
<td>Animal Science Division, Cooperative Extension Service, Agricultural Experiment Station, University of Nevada, Max C. Fleischmann College of Agriculture, Reno, Nevada</td>
<td>Hatch-43</td>
<td>R. L. Taylor, Donald Marble, M. D. Reynolds</td>
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### CURRENT RESEARCH PROJECTS IN THE UNITED STATES (Cont.)

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<tr>
<td>1. Influence of Spleen on Bovine Anaplasmosis</td>
<td>College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma</td>
<td>National Institutes of Health</td>
<td>E. W. Jones, G. B. Klaus</td>
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<tr>
<td>2. Immunization and Treatment of Cattle</td>
<td>College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma</td>
<td>State</td>
<td>C. C. Pearson, I. O. Kliewer</td>
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<td>3. Pathology of Anaplasmosis in Cattle</td>
<td>College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma</td>
<td>State</td>
<td>W. E. Brock, G. B. Klaus, I. O. Kliewer</td>
</tr>
<tr>
<td>1. The Investigation of the Biochemical Factors Affecting <em>Anaplasma marginale</em></td>
<td>Animal Disease Department, Georgia Coastal Plain Experiment Station, University of Georgia, College of Agriculture, Tifton, Georgia</td>
<td>State</td>
<td>James G. Miller, Walter Wilkinson, Jerry Gaskins</td>
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<tr>
<td>2. The Examination of 270 White-Tailed Deer (Odocoileus Virginianus) from Anaplasmosis Enzootic Areas of Southeastern United States for Evidence of Anaplasmosis</td>
<td>Animal Disease Department, Georgia Coastal Plain Experiment Station, University of Georgia, College of Agriculture, Tifton, Georgia</td>
<td>State</td>
<td>James G. Miller, Walter Wilkinson, Jerry Gaskins</td>
</tr>
<tr>
<td>Anaplasmosis of Cattle: Its Diagnosis, Characteristics, and Relationship to Other Arthropod-Borne Diseases</td>
<td>Department of Veterinary Science, University of Florida, Gainesville, Florida</td>
<td>Agricultural Experiment Station and U. S. Atomic Energy Commission</td>
<td>George Edds, Frank White, Charles Simpson</td>
</tr>
</tbody>
</table>
ECONOMIC LOSSES CAUSED BY ANAPLASMOSIS

The extent of economic losses caused by anaplasmosis was estimated recently to average $35,000,000 per year. There is no new information available which would indicate that this estimate should be revised, either upward or downward. If and when a mandatory reporting system is established on a national basis, the estimate can be realistically evaluated.

The incidence of clinical cases of anaplasmosis is not a reliable estimate of the extent of Anaplasma infection within the cattle population. Animals which harbor the causative agent, but which do not manifest clinical signs, can be and are being detected by serologic tests. There are a number of public-supported laboratories conducting complement-fixation tests for anaplasmosis throughout the United States. Also, private laboratories are conducting capillary agglutination tube tests to an unknown extent.

RECOMMENDATIONS OF THE COMMITTEE ON ANAPLASMOSIS

1. Economic Loss Survey: It is recommended that the Anaplasmosis Committee of the United States Livestock Sanitary Association again sponsor a survey of estimated economic losses resulting from anaplasmosis. This should be done through State Departments of Agriculture with the assistance of veterinary associations and cooperating animal disease control agencies. This survey should be completed by October, 1967.

2. Reporting Serological Test Results: It is recommended that the Animal Health Division, ARS, USDA, assemble data covering the anaplasmosis-testing activities of all laboratories conducting tests for this disease. A uniform reporting system should be devised, and the summarized data should be published regularly for the information of all concerned. The support of private laboratories and the livestock industry should be sought to this end. To avoid the possibility that anaplasmosis testing would "go underground," the identity of the animals tested should not be an integral part of the reporting system. The United States Livestock Sanitary Association should lead efforts to obtain the necessary funds to carry out the reporting activity.

3. Selecting Cattle of Appropriate Age to Study Immunogenic Efficacy of Anaplasma Vaccine: It has been known for many years and it is now an accepted fact that the severity of bovine anaplasmosis is directly related to the age of the animal. Namely, the severity of the disease increases as the age of the affected animal increases. Economically, anaplasmosis is most important in animals older than two years. Thus, for an objective evaluation of immunogenicity of a given vaccine, susceptible cattle not younger, and probably older, than two years should be used.

4. Development of Vaccines: Ideally, the best vaccine would be one which would protect animals from Anaplasma infection, thus eliminating the increase of Anaplasma carriers. All promising avenues of vaccine
research should continue to explore toward accomplishment of this goal. In the meantime, research should continue to fully evaluate the present vaccine and also encourage research toward development of other promising vaccines.

5. *Standardized Challenge Dose:* It is recommended that a sub-committee of the Anaplasmosis Committee be appointed to study the standardization of the challenge dose of infectious material to be used in immunization trials.

6. *Wild Mammalian Hosts:* Studies should be intensified in all areas of the United States where anaplasmosis is enzootic to determine the role of wild mammals in the maintenance of the infection in nature. This problem should be approached by inoculating blood of wild ruminants such as deer and antelope into susceptible calves. Since serology has been shown to be unreliable as an indicator of latent Anaplasma infection in deer and other wild ruminants, CA test application should be explored further. Also, various small wild mammals, which are normally hosts for immature stages of ticks that attack cattle as adults, should be similarly checked for the presence of Anaplasma infection. These studies are necessary to establish a complete picture of the epizootiology of anaplasmosis in various parts of the country.

7. *Vectors:* The role of ticks and other vectors should be investigated to determine the mode of survival and propagation of Anaplasma in invertebrate hosts. This should include continuation of studies such as those with fluorescent antibody technique on ticks, and those which use tick tissue cultures as a possible means of propagating the organism in the laboratory.

8. *Chemotherapeutics:* Efforts should be continued to develop new chemotherapeutics which can be used to efficiently and economically destroy the Anaplasma carrier stage. When a new compound is detected which has a reasonable affect on Anaplasma, then this compound should be further studied through basic research, and modified in a way suitable for obtaining the maximal desired effect against Anaplasma.

9. *Capillary Agglutination Tube Test:* It is recommended that the capillary agglutination (CA) tube test performed with antigen tested and approved by the Veterinary Biologics Division of the United States Department of Agriculture, in addition to the complement-fixation (CF) test, be recognized as an official test for determining the anaplasmosis status of an animal and that the official use of both tests be upon approval of each state animal health official.

10. *Pilot Field Trials:* It is recommended that money be made available in the U. S. Department of Agriculture for pilot field trials in key states to study the feasibility of control and eradication of anaplasmosis, using the present tools now available. Such studies should continue for no less than five years.
REFERENCES

21. Report #51 from Dr. T. E. Franklin, School of Veterinary Medicine, Texas A&M College Station, Texas.
23. Report #8 from Dr. C. M. Hibbs, Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas.
24. Report #9 from Dr. K. L. Kuttler, Veterinary Science Department, University of Nevada, Reno, Nevada.
25. Report #10 from Dr. J. W. Osebold, School of Veterinary Medicine, University of California, Davis, California.
26. Report #12 from Dr. J. W. Scales, Head, Animal Disease Department, Agricultural Experiment Station, Mississippi State University, State College, Mississippi.
27. Report #7 from Mr. W. G. Hepworth, Director, Game and Fish Research Laboratory, University of Wyoming, Laramie, Wyoming.
28. Report #4 from Drs. L. E. Foote and E. E. Roth, Department of Veterinary Science, Louisiana State University, Baton Rouge, Louisiana.
47. Ristic, M. and associates: Coll. of Veterinary Medicine, University of Illinois, Urbana, Illinois. (Unpublished data).
REPORT OF COMMITTEE

THE LEUCOCYTE COUNTS OF CATTLE ABORTING DUE TO 
BRUCELLA ABORTUS INFECTION COMPARED TO 
LEUCOCYTE COUNTS OF NONINFECTED 
CATTLE CALVING NORMALLY 

B. W. Bierer, V.M.D., and H. S. Powell, D.V.M.* 

Columbia, South Carolina 

The leucocyte count is valuable as a diagnostic aid in certain diseases of cattle. In human infections due to Brucella abortus, the leucocyte count has been described as an aid to differential diagnosis.

The use of the electronic cell counter in making leucocyte determinations has become routine in some diagnostic laboratories. Its use furnishes a method for rapid enumeration of white blood cells.

The purpose of this report is to summarize results obtained from conducting leucocyte counts on 77 cattle exposed to a virulent strain of Brucella abortus. The counts were determined at regular intervals over a period of more than two years. Only the results obtained just prior to and immediately following parturition are reported.

MATERIALS AND METHODS

The counts were conducted on heparinized blood drawn from the jugular vein (two drops one percent heparin solution per five milliliters).

Blood was collected from each individual at weekly intervals for four consecutive weeks prior to and for four consecutive weeks following normal calving or abortion. Cell counts were made with an electronic cell counter within three hours of the time of collection.

RESULTS

The average leucocyte count of 37 aborting infected cattle approximately four weeks prior to abortion was 10,200/cubic millimeter. On 32 noninfected cows approximately four weeks prior to normal parturition, the counts averaged 9,200/cmm. There were eight infected animals which delivered a full term live calf (normal-calving infected). Their average count at approximately four weeks prior to calving was 9,700/cmm.

The average leucocyte counts of all 77 cattle on succeeding weeks up to the time of calving and for four weeks following calving are recorded (Table I).

*Associate Laboratory Director, Clemson University Livestock-Poultry Health Department.
**Brucella abortus strain 2308.
***Brucellosis Project conducted at the Clemson University Livestock-Poultry Health Department to be reported to the Brucellosis Committee, United States Livestock Sanitary Association, 1966 Annual meeting.
****Coulter Counter, Model A, Coulter Electronics, Hialeah, Florida.
TABLE I
Average Leucocyte Counts (Hundreds Per Cubic Millimeter)

<table>
<thead>
<tr>
<th>No. Cows</th>
<th>Preparturient</th>
<th>Postparturient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4  3  2  1</td>
<td>1  2  3  4</td>
</tr>
<tr>
<td>Aborted Infected</td>
<td>102 97 92 90</td>
<td>79 87 93 93</td>
</tr>
<tr>
<td>37</td>
<td></td>
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<tr>
<td>Normal Calving Noninfected</td>
<td>92 94 96 100</td>
<td>89 97 93 92</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
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<tr>
<td>Normal Calving Infected</td>
<td>97 94 101 102</td>
<td>80 93 87 94</td>
</tr>
<tr>
<td>8</td>
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TABLE II
Normal Calving Noninfected (Hundreds Per Cubic Millimeter)

<table>
<thead>
<tr>
<th>No. Cows</th>
<th>Preparturient</th>
<th>Within 24 Hours</th>
<th>Postparturient</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Weeks</td>
<td>Parturition</td>
<td>Weeks</td>
</tr>
<tr>
<td></td>
<td>3  2  1</td>
<td></td>
<td>1  2  3</td>
</tr>
<tr>
<td>8</td>
<td>98 101 104</td>
<td>154 96</td>
<td>93 94 98</td>
</tr>
</tbody>
</table>

Average leucocyte counts on eight individuals taken at time of parturition are given in Table II. The average leucocyte count was found to increase from 10,400/cmm. one week before, to 15,400/cmm. at time of parturition. The total leucocyte count dropped off very sharply within the next 24 hours to an average of 9,600/cmm.

SUMMARY AND CONCLUSION

There was a definite pattern recognized in the counts following parturition. This pattern was observed in both aborting and normal calving animals (Table I). A sharp drop in the first weekly post-partum count was noted in all groups.

There was a gradual decrease in the average leucocyte count in the aborted infected group, beginning four weeks prior to abortion and extending up to time of abortion.

In the normal-calving noninfected group, the pre-calving counts remained stable up to the time of calving. The sharp drop in the total leucocyte count (similar to that observed in aborted infected group) was observed at the first weekly post-partum sampling.

The numbers of normal calving infected cows were small (eight head), but a similar sharp drop in the count was also noted in this group.

The transient increase in total white cell count at time of parturition (Table II) is described by Selye\textsuperscript{4} as a manifestation characteristic of the
alarm reaction in his general adaptation syndrome. Differential counts* made on blood smears from these eight animals showed that this increase was the result of a neutrophilic leucocytosis. Selye states that this neutrophilic leucocytosis is non-specific and can be produced by such diverse alarming stimuli as adrenaline, formaldehyde, cold, trauma or forced muscular exercise.

Based on the above findings we could find no changes in total white counts which could be specifically attributed to infection with *Brucella abortus* strain 2308.

REFERENCES


*Biological Department, Wisconsin Alumni Research Foundation, Madison, Wisconsin.*
A TREND IN THE PREVALENCE OF BRUCELLOSIS IN IOWA SWINE

Stanley L. Hendricks, D.V.M.* and William J. Hausler, Jr., Ph.D.**

In 1960 agglutination tests of blood specimens from a random sample of 3,345 Iowa hogs revealed that 83 reacted positively to brucella antigen in dilutions of 1:80 or higher. That study was stimulated by a sudden outbreak of brucellosis which began in 1959 among employees of a swine slaughtering plant. The incidence of brucella infection among workers in the plant remained at an unusually high level with some fluctuation through 1964 and gradually decreased in 1965 with eleven cases recorded. During the first eight months of 1966 only two cases were reported from this plant.

The reported incidence of human brucellosis for the entire state of Iowa declined markedly during the 1956-1965 period (Table I). Undoubtedly a portion of this reduction was due to the accelerated bovine brucellosis eradication program of the Iowa and United States Department of Agriculture, which resulted in Iowa being designated as a Modified Certified Brucellosis Area on January 11, 1966. Epidemiologic studies in Iowa have shown that a high percentage of human brucellosis cases in recent years has been due to Brucella suis infection in hogs.

The apparent cessation of the outbreak in the swine slaughtering plant and the decrease in human cases reported in the entire state stimulated the study reported herein.

MATERIALS AND METHODS

Blood specimens were collected while the hogs were being exsanguinated at slaughter in the same two plants in which the 1960 studies were

TABLE I

| Reported Cases of Human Brucellosis in Iowa 1956-1965 |
|---------|---------|
| 1956    | 360     |
| 1957    | 214     |
| 1958    | 382     |
| 1959    | 361     |
| 1960    | 379     |
| 1961    | 219     |
| 1962    | 105     |
| 1963    | 155     |
| 1964    | 114     |
| 1965    | 78      |

*Director, Division of Veterinary Public Health, Iowa State Department of Health, Des Moines, Iowa.

**Director, State Hygienic Laboratory, University of Iowa, Iowa City, Iowa.
PREVALENCE OF BRUCELLOSIS IN IOWA SWINE

111

done. Since these plants are geographically separated by 350 miles and because of marketing patterns, the hogs slaughtered in the plant in eastern Iowa for the most part are from a different swine growing area than those killed in the western Iowa plant. In the eastern Iowa plant (the plant in which the outbreak occurred) approximately 200 specimens were collected during each of two days in April, two days in May and five days in June, 1966. Specimens were taken from every tenth butcher hog or every fourth sow until the desired number was obtained each day. In the western Iowa plant approximately 250 specimens were taken at random during each of eight days between July 20 and August 4, 1966. Blood was taken from a larger proportion of sows than from butcher hogs in an attempt to obtain a meaningful number of sow specimens. The specimens were delivered to the laboratory within twenty-four hours of collection by parcel post or air express.

In the laboratory the serum was removed from the clot and inactivated for thirty minutes in a 56°C. water bath. Rapid plate antigen and tube antigen supplied by the Animal Health Division, United States Department of Agriculture,* and used routinely in human serum agglutinations, were used on the hog sera. Each undiluted serum specimen was screened by the rapid plate method and all positive specimens were then titrated by the tube-agglutination technique. The tests were incubated four hours in a 37°C. water bath, eighteen hours at 4°C., and one hour at room temperature prior to recording the titer.

RESULTS AND DISCUSSION

The results of the tests are shown in Table II. It is readily noted that only five of a total of 3,884 specimens were positive in dilutions of 1:80 or higher. This is in contrast to 83 specimens found positive in the same dilution among 3,345 tested in 1960,1 and suggests a marked reduction in prevalence of brucellosis in Iowa hogs.

A review of results of routine specimens collected by practicing Iowa veterinarians and tested at the State-Federal Brucellosis Laboratory at Iowa State University indicates no comparable decline in reactor rate during the 1957-1965 period (Table III). It is not likely that the routine testing is a true indicator of the prevalence of the disease since the animals tested are selected for various reasons as reported previously1 and do not constitute a random sample. It is noted, however, that the number of hogs tested increased from 26,000 in 1956 to 216,000 in 1964. The decreases in 1960 and 1965 coincide with reduced hog populations in those years.

This great increase in numbers of specimens tested coincides with and undoubtedly is due to certain changes in Iowa law pertaining to swine brucellosis. A law requiring a negative brucellosis test on all boars sold for breeding purposes became effective July 1, 1961 and two years later

*Obtained through courtesy of Dr. Grant Blake, Animal Health Division, U.S. Department of Agriculture.
TABLE II

Results of Agglutination Tests of Blood Specimens from Swine from Two Areas in Iowa in 1966

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<tr>
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<td>Eastern Iowa</td>
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<tr>
<td>Butcher hog</td>
<td>1364</td>
<td>1350</td>
<td>6</td>
<td>5</td>
<td>3</td>
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<td>0</td>
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<tr>
<td>Sow</td>
<td>543</td>
<td>532</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Western Iowa</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Butcher hog</td>
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<td>1408</td>
<td>1</td>
<td>4</td>
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<tr>
<td>Sow</td>
<td>564</td>
<td>557</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Total</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Butcher hog</td>
<td>2777</td>
<td>2758</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sow</td>
<td>1107</td>
<td>1089</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total - all classes</td>
<td>3884</td>
<td>3847</td>
<td>15</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE III

Hogs Tested, Positive Reactors, and Validated Herds in Iowa 1956-1965

<table>
<thead>
<tr>
<th>Year</th>
<th>No. Tested*</th>
<th>Positive Reactors*</th>
<th>Validated Herds**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>1956</td>
<td>26,851</td>
<td>492</td>
<td>1.8</td>
</tr>
<tr>
<td>1957</td>
<td>30,953</td>
<td>277</td>
<td>0.9</td>
</tr>
<tr>
<td>1958</td>
<td>31,608</td>
<td>325</td>
<td>1.0</td>
</tr>
<tr>
<td>1959</td>
<td>40,786</td>
<td>572</td>
<td>1.4</td>
</tr>
<tr>
<td>1960</td>
<td>25,258</td>
<td>258</td>
<td>1.0</td>
</tr>
<tr>
<td>1961</td>
<td>76,284</td>
<td>542</td>
<td>0.7</td>
</tr>
<tr>
<td>1962</td>
<td>93,796</td>
<td>944</td>
<td>1.0</td>
</tr>
<tr>
<td>1963</td>
<td>141,182</td>
<td>1572</td>
<td>1.1</td>
</tr>
<tr>
<td>1964</td>
<td>216,590</td>
<td>2369</td>
<td>1.1</td>
</tr>
<tr>
<td>1965</td>
<td>187,548</td>
<td>1678</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data Source: Reference #1 and

**Dr. N. K. Kinney, Chief, Division of Animal Industry, Iowa Department of Agriculture, Des Moines, Iowa.

this requirement was expanded to include female breeding animals. Beginning July 1, 1965, herds of origin of reactivity breeding animals were placed under quarantine until the herd was slaughtered or until a subsequent test of the entire herd revealed no reactors. These law changes not only increased the number of animals tested but very likely influenced the selection of animals for test and in turn the positive reactor rate. The law which required the quarantining of herds of origin of reactor animals probably especially increased the positive reactor rate as it tended to concentrate the testing in herds which were identified as being infected. Thus the reactor rates for the different years are not comparable.
Another reason the rates are not comparable is because of a change in interpretation of test results. Prior to 1962 only animals reacting in a dilution of 1:100 or higher were classified as reactors. Since 1962 animals reacting in a 1:50 dilution also have been classified as reactors. This change undoubtedly is reflected in the increase in positive reactor rate from .7 percent in 1961 to one percent in 1962 (Table III).

The law changes also have stimulated swine growers' participation in the "Validated brucellosis-free swine herd" program as indicated by the increased number of validated herds (See Table III).

The fact that all the hogs that were positive at 1:80 or higher in the 1966 study were from the eastern Iowa (specifically E. Central) plant is of interest when compared with the geographic distribution of infected herds and lots revealed in routine testing in 1965 (See Figure A). A higher rate of infected herds and lots per 1,000 swine breeding farms was reported for the east central and south east districts than for other districts in the state.

CONCLUSIONS

The positive reactor rates among hogs slaughtered in two plants when compared with a similar survey in 1960 indicates a marked reduction of brucellosis in hogs in Iowa has occurred. This seems to be supported by a decrease in reported human brucellosis cases in the state. The positive reactor rate among hogs tested routinely at the State-Federal Brucellosis Laboratory probably does not present a true picture of the prevalence of swine brucellosis because of several factors.

SUMMARY

Brucellosis agglutination tests applied to 3,884 blood specimens collected at random at time of slaughter in two Iowa plants revealed five positive reactors in dilutions of 1:80 or higher. Comparison with a similar survey in 1960 suggests there has been a marked reduction of swine brucellosis in the state. During the same period there has been a considerable reduction in reported human brucellosis cases in Iowa. Because of various factors, the reactor rate among hog blood specimens tested routinely at the State-Federal Brucellosis Laboratory does not seem to be a true indicator of the prevalence of swine brucellosis in Iowa.

ACKNOWLEDGMENT

Assistance by the Sioux City Health Department and the Meat Inspection Division of the U. S. Department of Agriculture in collection of specimens is gratefully acknowledged.
Figure A. Numbers of Infected Herds and Lots Per 1,000 Swine Breeding Farms as Disclosed by Routine Testing in 1965.

Data Source: Geographic distribution of infected herds and lots from Grant E. Blake, D.V.M., Animal Health Division, ARS, USDA, Des Moines. Swine breeding farms from State Farm Census Supplement No. 3, Iowa Crop and Livestock Reporting Service.
REFERENCES


Encouraging progress has been made in the eradication of brucellosis during the past year. It is not, however, reflected in the number of certified areas established compared to previous years. The progress is apparent in the reduced infection rate at a time when much of the testing is done in herds suspected of being infected as a result of screening tests. The encouraging aspect is that at the present time only 140 counties remain that have not inaugurated an area eradication program (Figure 1). There is no doubt that one of the factors stimulating this increase in area work is the action taken by this Association last year when it was recommended that, "The Federal Government amend the regulations governing the interstate movement of livestock to provide that effective January 1, 1968, cattle moving into Modified Certified Brucellosis Areas and Certified Brucellosis-Free areas must originate from Modified Certified Areas."
Brucellosis Areas or Certified Brucellosis-Free Areas. It is further recommended that each State adopt similar regulations controlling intra-state movement of cattle."

The urgency of complete eradication of brucellosis in all species is also emphasized by the availability of funds for support of the program at this time. A few years ago representatives of this organization presented factual history, very realistic projected program plans and their resultant benefits to the Congress, gaining adequate support to conduct the program at a level which would have permitted the entire Nation to be modified certified by this time. The Congress has been considerate; however, there is now some concern among that body that the projected goal has not been attained. There has been a continuing progress in the eradication of brucellosis, but this is to a great extent because of the efforts of Modified Certified Brucellosis Areas to achieve Certified Brucellosis-Free status.

The continuing high incidence of the disease in some areas is causing increased costs for modified certified areas in their efforts to eradicate brucellosis. With the rapid transportation of livestock today, brucellosis can be moved very quickly from the unknown areas to the free areas.

CERTIFICATION

The specific examples of progress are seen in the fact that today over 89 percent of the Nation's counties are certified (Figure 2). They are either Modified Certified or Certified Brucellosis-Free Areas. During
the last fiscal year, two states—Massachusetts and Nevada—were successful in attaining Certified Brucellosis-Free status, making nine States and the Virgin Islands certified-free. Certification of Massachusetts completed the entire New England area as a block of certified-free States. There were 208 counties that achieved free status. This is 20 percent greater than the previous year.

Iowa was the only State to reach modified certified status during the year, becoming the 38th State to attain modified certified status. There were 78 counties initially certified—27 of these were in Iowa. Iowa is an excellent example of what can be accomplished in brucellosis eradication. There had been little progress in the program until July 1963. At that time, there was an all-out effort by the industry in Iowa to complete certification. That goal was achieved in January 1966.

Currently, there are 718 free counties, 22 percent of the Nation's total (Figure 3). These contain about one-half million herds and over 11 million cattle.

Twenty-nine States and Puerto Rico are not modified certified. There are 2,107 modified certified counties, 67 percent of all counties. There are 188 counties or seven percent conducting area work, leaving 140 or four percent remaining to inaugurate area activity.

### Brucellosis Eradication

#### COUNTY CERTIFICATION STATUS

<table>
<thead>
<tr>
<th>Percent of U.S. Counties</th>
<th>June 30, 1965</th>
<th>June 30, 1966</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified Free</td>
<td>9%</td>
<td>7%</td>
</tr>
<tr>
<td>Modified Certified</td>
<td>15%</td>
<td>22%</td>
</tr>
<tr>
<td>Area Work in Progress</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Individual Herd Participation</td>
<td>72%</td>
<td>67%</td>
</tr>
</tbody>
</table>

Figure 3
Another indication of progress being made is the decrease in the percentage of herds found suspicious to the ring test (Figure 4). For two years, this had leveled at 9/10 of one percent; but this year there was a drop to 7/10 of one percent found suspicious.

The BRT is still our best method for surveillance of dairy herds. As the incidence of brucellosis is reduced, States should endeavor to increase ring testing so each herd would be sampled quarterly (Figure 5). Twenty-four States are now conducting four rounds per year. Twenty-two, three and four States are still conducting the BRT only twice annually. The benefits of the BRT must be recognized. It is a relatively inexpensive means for screening millions of cattle. Furthermore, it usually eliminates the problem of residual vaccination titers seen in blood tested herds; and with the regularity of screening quarterly, the BRT can locate infection before it has had an opportunity to spread widely within the herd or to other herds.

Laboratories conducting the test should constantly be alert for any changes in the preservatives used for preserving the Babcock sample. Last year it was mentioned that dichromate compounds were being used.
These do affect the agglutinins present in milk from infected cows and infection can be missed. There should be a continuing surveillance of products used in each area to assure that the preservative used is mercuric chloride.

As dairy herds become fewer and larger, the sensitivity of the test must also be adjusted. Work in cooperation with the State of California has shown that this can readily be accomplished by adjusting the antigen/milk ratio. As the number of milking cows increase, the volume of milk in the test is increased accordingly. Results indicate that by this adjustment infection is disclosed which otherwise would have been missed.

MARKET CATTLE TESTING

The continued growth of the market cattle testing program is encouraging. Each year it becomes a more vital part of the brucellosis eradication program. This year there were 5,795,902 cattle backtagged—an increase of 47 percent over the number of animals backtagged last
year. Blood samples were collected from over three million of these. Last year, this Association recommended that market testing of dairy cows for brucellosis be discontinued. This recommendation was put into effect in January. However, in some areas of the country it became apparent that there were large numbers of cattle which could not accurately be classified specifically as beef or dairy at the packing plant. In order that the effective surveillance be maintained in these areas, it was necessary that samples again be collected from all backtagged cattle unless it could be determined that the cattle were dairy cattle. Plans have been made to correct this situation by use of a reversible tag, bearing the same identifying number on each side, with only a different background color. The tag can be applied to cattle covered by BRT with the yellow side up and to non-BRT cattle with the white side up, indicating a blood sample should be collected at the packing plant. This should provide the means for correction of the duplicate testing of dairy cattle and also provide identification of the animal for the tuberculosis eradication program. The Livestock Slaughter Division of Consumer and Marketing Service is urging each of its field stations to cooperate to the fullest extent possible. This cooperation is greatly appreciated and should make possible a continuing increase in the percentage of blood samples collected from backtagged cattle.

During the last fiscal year, MCT accounted for over 42 percent of all blood samples tested (Figure 6). Of the 4,927,385 samples drawn, 47,630 were reactors. This is the first time that the incidence rate in this class of cattle dropped below one percent.

As a result of traceback of MCT reactors, 4,173 herds of origin were tested. These herds revealed 19,142 additional reactors. Although only about one of these herds of origin are found infected, when infection is sound, it is frequently high. This year the infected herds found on MCT traceback averaged over 10 percent animal infection within the herd.

In view of the savings of manpower that could result from a fully effective MCT program, States are urged to develop complete MCT capabilities for use in both initial and recertification of areas. When adequate coverage is attained on a Statewide basis, certified status can be maintained on a State rather than county basis. The State can remain certified, without the recurring anniversary periods as recommended at last year's meeting. The reduced record keeping and reporting associated with this system should reduce the clerical work relating to MCT. Kansas and Missouri have been on a Statewide program for a year with apparent satisfactory results. As a matter of interest, 23 States are annually testing over 10 percent of their adult cattle population in the MCT program. In the first year of operation, Missouri tested over 11 percent of their adult cattle population under the MCT program.

BRUCELLOSIS PROBLEM HERD

In 1959 procedures were developed and work inaugurated to eradicate brucellosis from those herds which had been infected for relatively long
periods. They were spoken of as problem herds and the program—the problem herd program. Training programs to provide specialized training for selected State and Division veterinarians have been conducted annually. In addition, there are eight veterinarians in the field having completed postgraduate training in brucellosis epidemiology; and five are presently enrolled in intensive study to that end, making a total of 33 specially trained brucellosis epidemiologists. Today, as a result of progress in eradicating brucellosis in many areas, every infected herd is studied in detail by a brucellosis epidemiologist. This is a vital part of maintaining a free area and a valuable aid in the modified area becoming free.

The epidemiologist's study of situations in the field is providing valuable data for program review. Infection in the certified-free areas is one of the subjects which has caused concern (Figure 7). At the close of the fiscal year, there were 667 certified-free areas. Of these, 56 percent have had no infection since they achieved free status, some of these were certified-free in 1960. Twenty-three percent of the counties had only singleton reactor herds; that is, herds which had a negative history—a single reactor was disclosed on one test and subsequent tests have been negative. One percent had two reactors on a single test and, subsequently, had negative herd tests. Three percent had both singleton and low titer reactor herds on a single test. Seventeen percent had multiple reactions.
BRUCELLOSIS-FREE COUNTIES

Revealing Reactor Herds

Total Free Counties 667

US DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

Figure 7

REACTOR HERDS FOUND
In Certified Brucellosis-Free Counties

US DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

Figure 8

72% of Reactor Herds disclosed on ONLY 1 TEST!
A review of the reactor herds in these free counties (Figure 8) reveals that 64 percent had a reactor on only a single test, eight percent had two reactors on only a single test or a total of 72 percent of the reactor herds in free areas disclosed reactors on only one test.

Another fact that has become increasingly apparent, from reports of the studies made by the epidemiologists is that one cause for recurring infection in herds was that the herd had not truly been freed of the disease. Some of the animals were still in the incubation period after exposure at the time they were released from quarantine. Several States have taken action to extend the quarantine period, requiring a second negative test prior to release. A study of blood titers of 20 nonvaccinated heifers after a controlled exposure revealed (Figure 9) that 30 days after exposure there were 14 reactors, three suspects and three remaining negative to the Standard Tube Test.

The picture in vaccinated cattle is even more alarming (Figure 10). At 30 days after exposure, there were no reactors and two suspects—12 were negative. All of these animals were subsequently confirmed infected by recovery of Brucella abortus from milk or tissues. It would appear that the spread of brucellosis from an infected herd to other herds cannot be prevented if quarantine is released on a single herd test revealing no reactors 30 days after removal of a reactor. A second test 60 days later would prevent that spread in most instances.

Brucellosis Eradication

**DEVELOPMENT OF TITERS**

*In Infected Non-vaccinated Cattle*

![Graph showing development of titers in infected, non-vaccinated cattle.](image-url)
In spite of efforts to increase calf vaccination in areas where the incidence of brucellosis is still high, there was a decrease of calves vaccinated in these areas. During the year, 6,657,036 calves were officially vaccinated.

The Brucellosis Committee has in previous years recommended that efforts be made to determine whether calves could be vaccinated at an earlier age without dropping the protection rate below the 60-70 percent level of protection afforded the calf vaccinated at six months of age. Two studies were completed this year and have been previously reported to the Brucellosis Committee at this session which indicate that the vaccination age can safely be lowered. This should alleviate to some extent the problem of residual titers resulting from calf vaccination.

For many years, *Brucella abortus* strain 45/20 has been under extensive study in England and other parts of Europe as an immunizing agent. This strain has one highly desirable characteristic in that it is non-agglutinogenic. Because of its virulence, further study of its immunogenic capabilities were discontinued when Strain 19 became available. The problem of residual titers from Strain 19, however, creates a renewed interest in 45/20 used as a bacterin.

Controlled studies conducted at Compton, England, indicate that when
45/20 bacterin is administered in two doses a resistance comparable to that produced by Strain 19 vaccine is achieved. A limited trial of a 45/20 bacterin was conducted in conjunction with the vaccination age study at South Carolina. Results there indicate that the bacterin given in two doses at the sixth and ninth month induces a resistance comparable to that of Strain 19. No work has been reported on the duration of the increased resistance inferred by the bacterin; the producer of the bacterin, however, recommends annual revaccination. Additional studies are necessary before a satisfactory evaluation can be made of this type of product.

One serious disadvantage of use of the bacterin in this country is the fact that the two doses of bacterin must be given at relatively precise intervals for optimum resistance.

**SWINE BRUCELLOSIS**

Nevada and the Virgin Islands achieved Validated Brucellosis-Free status during the year—Nevada, Utah, Vermont and the Virgin Islands are free of both bovine and swine brucellosis (Figure 11). Currently, there are 125 validated counties in eight States and the Virgin Islands and 2,265 validated herds. The interest in the eradication of brucellosis from swine has not been as great as anticipated. For several years, the Public Health Service has incriminated contact with infected swine as the cause of 2/3

**STATES WITH VALIDATED BRUCELLOSIS - FREE SWINE HERDS**

![Map of States with Validated Brucellosis-Free Swine Herds]

*Figure 11*
of the human cases of the disease. Another factor which the swine industry apparently overlooks is the loss of export markets for pork products. West Germany applied restrictions on pork imports January 1, 1966, requiring that the product be derived from swine originating in areas which were free of the disease and which had not been exposed within 90 days of slaughter. The Department successfully negotiated a temporary relaxation of that requirement; however, this will expire October 31, 1966. West Germany has advised there will be no extension of this relaxation.

When this becomes effective, it will amount to an annual loss of about $22.6 million in packers' sales of pork products. It can be expected that the packer has no recourse other than to pass that loss on to the producer. The other European countries will probably abide by their common agreement at a later date, and a much greater loss can be expected at that time.

In closing, it would be desirable to point out two additional means of measuring progress in the eradication of brucellosis. In past years some persons have pointed to the fact that there is still infection in modified certified areas, and it must be recognized that this is true. The goal of eradication has not yet been reached; however, a review of the data reveals that there are (Figure 12) about three times as many reactors found in the few non-certified States as there are in the entire certified area of the Nation.

**BRUCELLOSIS ERADICATION**

![Graph showing reactors found in 36 states and 14 states](image)

---

Figure 12
An equally dramatic example of progress can be seen in the location of infected herds found during recent years (Figure 13). In the last six years there has been a continuing drop in the number of infected herds found in the certified States, from over 22,000 in 1960 to about 8,000 in fiscal year 1966, whereas in the relatively small number of non-certified States, certified as of June 30, 1966, there has been a relatively constant number—slightly over or under 10,000 infected herds found annually.

These are examples of progress and certainly an indication that brucellosis can be eradicated. With a concerted effort, the goal of eradication can be achieved by 1975.
HOST-PARASITE RELATIONSHIPS IN BRUCELLOSIS

I. Reservoirs of Infection and Interhost Transmissibility of the Parasite*

Margaret E. Meyer, Ph.D.
Davis, California

The Hosts

All of the domesticated and semidomesticated animals used as livestock either are enzootically infected with brucellosis, or are known to be susceptible to this infection. Included among the livestock hosts are sheep, goats, cattle, yaks, bison, water buffalo, oxen, camels, reindeer, swine, and horses. In addition to these livestock, wild mammals also are known to serve as reservoirs of infection for this disease. Included among the wild mammals from which Brucella organisms have been isolated are various species of rodents, hares, fox, deer, moose, and elk. If the presence of a titer as determined by the serum agglutination test is considered to be incriminating evidence of susceptibility, then the spectrum of hosts may be even broader and include llama, vicuna, alpaca and possibly others.

The Parasite

Precise descriptions of the organisms that induce infection and accurate methods to determine their identification are prerequisites to understanding the epidemiology of infectious diseases. In a disease such as brucellosis, which has a variety of animals that can serve as hosts and reservoirs of infection, and which can be caused by several species of the causal agent, the fulfillment of these prerequisites is particularly important. With the recent additions of quantitative manometric techniques and of standardized Brucella bacteriophage strains to the conventional determinative methods, procedures now are available that ensure meticulous identification and differentiation of the microorganisms that constitute the genus Brucella.¹,²,³,⁴

Based upon these three methods of identification, the genus Brucella is classified into four species, B. abortus, B. suis, B. melitensis, and B. neotomae. Within the species there are subgroups that vary from the type species, which is always known as type 1 and which is regarded as having characteristics most typical of the species. These subgroups may vary from the type species in one or more biochemical characteristics (bio-types) or in serological characteristics (serotypes). However, all such subtypes are similar to the type species in a sufficient number of over all characteristics to be recognizable as a species member.

*From the Department of Veterinary Microbiology, School of Veterinary Medicine, University of California, Davis, California.
In the species *B. abortus* there are eight subtypes that vary from type 1 either in their serologic or in their conventional biochemical characteristics. However, all *B. abortus* subtypes display identical metabolic patterns as determined manometrically and all *Brucella* organisms that have the metabolic pattern of *B. abortus* are susceptible to lysis by the routine test dilution of *Brucella* phage, and all organisms having a metabolic pattern that differs from *B. abortus* are not susceptible to this phage activity.

*B. melitensis* contains three serotypes but no biochemical or metabolic variants.

*B. suis* has three subtypes that vary from the species description in biochemical features, metabolic pattern and/or serologically. The distinguishing and characteristic feature of all four biotypes of *B. suis* is their ability to oxidize L-arginine, DL-ornithine, and DL-citrulline.

*B. neotomae* contains no biotypes.

**Host-Parasite Relationships**

To ascertain the transmissibility of the various species of the parasite among and between the animals known to be susceptible to infection, 1,000 strains of *Brucella* that had been obtained from worldwide geographic sources and that included isolates from all reported hosts, were examined by each of the three recommended sets of methods to determine the species and biotype identity of the individual strains. These data then were analyzed to compare host of origin and tissue of origin of the isolate with its species and biotype identity. The results are summarized in Tables I and II.

The results of a similar strain identity-host of origin comparison previously have been presented on 550 *Brucella* strains. This present report includes those 550 strains plus an additional 450 isolates obtained from a wider range of hosts and from a wider range of geographic areas.

From the data on Table I it is clear that cattle are the preferential hosts of the species *B. abortus*, that sheep and goats are the preferential hosts of *B. melitensis* and that swine are the preferential hosts of *B. suis*. Each of these hosts can serve as a reservoir of infection for hosts of similar kind. However, the successful control of eradication of brucellosis also depends upon being able to assess the actual or potential threat of spread of the parasite among dissimilar hosts and also in being able to assess the hazard that wild animal reservoirs may pose to livestock.

**Susceptibility to *B. abortus***

Excluding the 30 strains of unknown host origin and the 35 isolated from human infections, only 36 isolates were from animals other than cattle. In areas where sheep are quartered closely with infected cattle, the sheep can become infected. However, experimental work now in progress indicates that strains of *B. abortus* that are capable of causing abortion in sheep are of an unusually high order of virulence. The more frequent outcome of exposure of sheep to *B. abortus* is excretion of the organism via the mammary gland.
TABLE I
Host of Origin and Species Identity of 1,000 Strains of Brucella

<table>
<thead>
<tr>
<th>Host of origin</th>
<th>Total number isolates examined</th>
<th>Species identity of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. abortus</td>
</tr>
<tr>
<td>Cattle</td>
<td>427</td>
<td>395</td>
</tr>
<tr>
<td>Sheep and goats</td>
<td>96</td>
<td>20</td>
</tr>
<tr>
<td>Swine</td>
<td>168</td>
<td>1</td>
</tr>
<tr>
<td>Horses</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Reindeer</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Bison</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Elk</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rodents</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Hares</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Foxes</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Camel</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Man</td>
<td>178</td>
<td>35</td>
</tr>
<tr>
<td>Dog</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>53</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
<td>496</td>
</tr>
</tbody>
</table>

TABLE II
Host Distribution of the Biotypes of Brucella suis

<table>
<thead>
<tr>
<th>Host of Origin</th>
<th>Total</th>
<th>B. suis type 1</th>
<th>B. suis type 2</th>
<th>B. suis type 3</th>
<th>B. suis type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>167</td>
<td>146</td>
<td>6</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Cattle (milk)</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sheep (ram)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Goats (lymph node)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hares</td>
<td>29</td>
<td>1</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rodents</td>
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<td>1</td>
<td>0</td>
<td>11</td>
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<td>12</td>
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<td>Man</td>
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</tr>
<tr>
<td>Dog</td>
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<td>1</td>
<td>0</td>
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<td>0</td>
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<tr>
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<td>34</td>
<td>79</td>
<td>18</td>
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</table>

Swine are apparently not susceptible to uterine infection with B. abortus. The literature records no instances of abortion in swine having been caused by this species of Brucella, and only on very few occasions has B. abortus been isolated from lymph nodes of hogs.

Bison inhabiting the great plains region of the United States are known to be infected with B. abortus. Where bison and cattle intermingle they could provide reservoirs of infection for each other.

Foxes have been found to be infected with B. abortus in Argentina and in parts of Europe. Available evidence indicates that the foxes became infected by ingesting fetuses. The role of the fox in transferring the
disease back to cattle is controversial, as it is not yet known whether infected foxes abort and/or excrete the organism.

Susceptibility to *B. melitensis*

Excluding those strains of unknown host origin and those isolated from human infections, only 32 strains of this species were isolated from hosts other than sheep and goats. Of the 29 strains isolated from cattle, only three were from fetuses and the remaining were recovered from milk. Since *B. melitensis* is not enzootic in the United States, all of these strains were obtained from countries other than the United States, primarily Malta and the mideastern countries. Even in these latter areas, where *B. melitensis* is enzootic in sheep and goats, abortion due to this organism is rare. However, cattle exposed to infected sheep and goats can become carriers and excrete the organism in their milk.

Swine are poor hosts for *B. melitensis* as there are no instances in which this species has been isolated from swine tissue. Organisms isolated from infected swine in the midwestern United States that were identified initially as *B. melitensis* are now known to be a biotype of *B. suis*?

Susceptibility to *B. suis*

Of the four *Brucella* species, *B. suis* is the most diverse in its biochemical and metabolic behavior, it being the only species wherein the biotypes display metabolic patterns that are not identical to the type species. Regarding the species as a whole, *B. suis* is found to be the cause of infection in a wider range of hosts than either *B. abortus* or *B. melitensis*. Since each of the biotypes within this species is associated with a different range of hosts and with different geographic regions, the data pertaining to *B. suis* are presented in detail on a separate table (II).

*B. suis*, Type 1

In all areas of the world where there are infected swine, *B. suis*, type 1 has been found. In these infected areas, *B. suis*, type 1 also has been isolated from rodents, rabbits, and hares. However, there is little or no evidence to suggest that *B. suis*, type 1 spreads from hare to hare or rodent to rodent. The occasional and individual rodent and hare found to be infected apparently are inadvertent transgressors into an area of infected swine, but rodents and hares then do not serve as a massive reservoir for the maintenance and spread of *B. suis*, type 1. The chain of infection is swine to swine, swine to hare and, under propitious circumstances, it could be spread from hare back to swine.

*B. suis*, type 1 has been recovered, though infrequently, from milk of cows exposed to infected swine. There are no literature reports incriminating *B. suis*, type 1 as a cause of cattle abortion, nor has this biotype ever been isolated from goat fetuses or goat milk.

*B. suis*, Type 2

*B. suis*, type 2 is enzootic and periodically epizootic in swine in western and central Europe. This biotype has been recovered from infected
swine in Denmark, Germany, Switzerland, Poland, Czechoslovakia, Romania, Bulgaria, and western Russia. In central and eastern Europe B. suis, type 2 is also enzootic in the wild hare, *Lepus timidus*. In contrast to *B. suis*, type 1, type 2 does spread from hare to hare and this animal provides a great reservoir of infection. There also exists unquestionable evidence\(^8\) that the infection spreads from the hare to swine. The chain of infection, therefore, can be swine to swine, swine to hare, hare to hare, hare to swine.

*B. suis*, type 2 has never been isolated from cattle tissues or fluids, has never been associated with human infection, and intensive investigations have failed to reveal the presence of this biotype in the western hemisphere or in Asia.

**B. suis, Type 3**

*B. suis*, type 3 shares some biochemical features with *B. melitensis* and is the type that initially was misidentified as *B. melitensis* of swine origin. It is enzootic in the swine population of midwestern United States but is not restricted to this area. Strains of this biotype have been recovered from swine in California, Argentina, Hong Kong and Malaysia. Interestingly, it has not been reported from Europe.

*B. suis*, type 3 also has been isolated from the rodent species of *Arvicanthis niloticus* (mouse) and *Mastomys natalensis* in Kenya; from the rodent species of *Rattus assimilis*, *Melomys cervinipes*, and *M. lutillus* in Australia, and from rodents in Thailand.

It is not yet known how great a threat rodents pose as a reservoir of infection for swine.

**B. suis, Type 4**

*B. suis*, type 4 is enzootic in the reindeer, *Rangifer tarandus*, in Siberia, Alaska, and Canada. This biotype of *B. suis* apparently was brought to Alaska from Siberia when these animals were imported from Siberia in 1891. The remarkable feature of *B. suis*, type 4 is that it is a cause of abortion in a ruminant animal and is transmissible among reindeer.

**B. neotomae**

Only eight strains of *B. neotomae* have been isolated and all of these were recovered from the wood rat, *Neotoma lepida*. As far as is known, this species is confined to this rodent and to the state of Utah.

From the information presented herein, it can be concluded that *Brucella* organisms are not readily transmissible from their preferential host to dissimilar hosts and that no serious or threatening reservoir of infection exists presently in wild animals in the United States. While there will probably continue to be unexplainable occurrences of infection in individual animals and in individual locales, never the less, it is reasonable to anticipate that eradication of brucellosis can be achieved with the means afforded by our present knowledge.


REPORT OF THE COMMITTEE ON BRUCELLOSIS


In presenting the report of the Brucellosis Committee of the United States Livestock Sanitary Association, may I first express my sincere thanks to the members of this Committee who gave up Monday afternoon and Tuesday morning to the open meeting discussions. These meetings were well attended by livestock personnel and regulatory officials. As in the past, everyone who desired to discuss a problem was given the opportunity to express himself to the fullest extent. The interest shown during these meetings and the discussions have been very helpful to the Committee in preparing this report. The Committee trusts that this interest will continue and with your advice and recommendations, our goal of brucellosis eradication will be attained.

Since our last meeting, 219 counties have qualified as Certified Brucellosis Free Areas. Also, one State, Iowa, achieved a Modified Certified status, and two States, Massachusetts and Nevada, attained a Certified Brucellosis-Free status. At this time 38 States have achieved the Modified Certified status. Of these, nine States and the Virgin Islands have gone on to reach the ultimate goal, a Brucellosis-Free status. There are now a total of 718 free counties.

OFFICIAL VACCINATES

The Committee strongly recommends that heifer calves be vaccinated as near four months of age as possible because this procedure has the advantages of 1) practically eliminating significant residual vaccinal titers at breeding age, 2) all of the research evidence shows that immunity induced in the animals vaccinated at younger ages is equal to that induced in the animals vaccinated at older ages.

Recent research has revealed that the immunity induced in heifer calves vaccinated at three months of age with Strain 19 was equivalent to that in heifers vaccinated older than this age. Furthermore, there is limited evidence that a servicable immunity can be induced in heifer calves vaccinated as young as two months of age.

Therefore this Committee recommends that the minimum official vaccination age be lowered from four to three months.

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RECOMMENDED AGE FOR TESTING OFFICIAL VACCINATES

The Committee reviewed comments received from the beef cattle industry relative to the age "limits" that official vaccinates should be initially tested. In view of the Committee's recommendation that heifer calves can be officially vaccinated as young as three months of age, the lowering of the initial testing age of official vaccinates should become effective at a reasonable date in the future. The Committee believes that the proposed date of January 1, 1970 is a reasonable one because it gives the cattle industry ample time to initiate and carry out a program of younger age vaccination. If this program is begun immediately, lowering the initial testing age of official vaccinates would not cause a hardship for the cattle owner, since heifers vaccinated before July 1, 1967 will not be affected by the proposed effective date. Moreover, this regulation will facilitate the removal of most pregnant infected vaccinated heifers from circulation before they have an opportunity to spread brucellosis.

In the interest of sound brucellosis eradication procedures, the Committee felt obligated to recommend that 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, after January 1, 1970.

LENGTH OF QUARANTINE

In the light of recent information from the field relative to infected herds, it is evident that the incubation period is frequently greater than 30 days. It is therefore recommended that an infected herd be maintained in quarantine until tested negative not less than 60 days after removal of the reactor animal. The Committee strongly recommends that each State take the necessary action to comply with this recommendation.

INTERSTATE MOVEMENT OF CATTLE

The Committee again expressed its concern about the hazards associated with movements of cattle from non-certified areas. We commend the non-certified States for the progress that has been made by the counties in these areas. We encourage the remaining non-certified counties to intensify their efforts to achieve a Modified Certified Brucellosis status by January 1, 1968.

CARD TEST

The card test was approved at the 1965 meeting as a screening procedure pending the receipt of additional data on the results of use of the test. Reports of use of the card test in the field as well as controlled studies were presented for review. State officials from several non-certified States indicated that use of this procedure was the only method which could provide the means for achieving Modified Certified Brucellosis status by January 1, 1968. On the basis of the foregoing information, the
Committee recommends that the card test be approved as an official test for Brucellosis.

This test is to be applied only by authorized personnel under direction of the State Veterinarian. Each test performed must be reported to the State Veterinarian immediately for permanent record accompanied by the supporting test card.

INDIAN RESERVATIONS AND NATIONAL PARKS

This Committee recommends that all cattle and Bison individually owned by Indians and/or Tribal or Federal controlled, be immediately brought into compliance with State and Federal regulations as prescribed in the Uniform Methods and Rules for the control and eradication of brucellosis in domestic cattle.

BRUCELLOSIS IN BISON

Whereas, the present Federal Law and Regulations pertaining to the control and eradication of Brucellosis have been developed over many years, based on the cooperation of the states and the State cattlemen.

Whereas, the grazing of Bison is becoming more frequent and Bison are increasing in numbers.

Whereas, the management of a herd of Bison is in many ways different than the management of a herd of cattle.

Whereas, the control of Brucellosis is important in the herds of Bison in this country.

Therefore, be it resolved: that a program for the control of brucellosis in Bison be established that would certify Bison herds according to the Uniform Methods and Rules for domestic cattle.

STRAIN 19

There have been requests by State officials that there be a reevaluation of the maximum and minimum dosage of *Brucella abortus* Strain 19 vaccine necessary to produce a serviceable resistance to the disease. In the interval it is recommended that vaccine purchased from public funds have a maximum viability count of not more than 15 billion and not less than 10 billion *Brucella abortus* Strain 19 organisms per M.L. and that further research be conducted to determine the minimum number of organisms required to provide a servicable resistance.

SWINE BRUCELLOSIS

Swine Brucellosis eradication is not progressing as rapidly as the bovine brucellosis eradication program.

The loss of foreign markets because of the presence of Brucellosis in our swine herds is of economic significance.

It is recommended that:
1. Uniform methods and Rules for Porcine brucellosis eradication be applied in all States.

2. Federal regulations be considered to require on or after January 1, 1968 breeding swine moved interstate be from Validated Brucellosis Free herds only.

3. All breeding swine consigned to slaughter be identified and tested for brucellosis.

4. The United States Department of Agriculture and the States provide indemnity funds for swine found to be infected or exposed to brucellosis.

5. State and Federal funds be provided to augment these recommendations. It is further recommended that the swine industry organizations be encouraged to establish swine brucellosis committees to make recommendations to the Brucellosis Committee of the United States Livestock Sanitary Association.

CERTIFICATION TESTING PERIOD

There has been a difficulty in completing the blood testing of the herds not adequately screened by BRT or Market Cattle Testing within the 18 month period now prescribed for establishing Certified Brucellosis Free Areas. It is therefore the recommendation of the Committee that the period of time for the blood testing of herds not adequately screened be not less than 18 months nor more than 24 months for purposes of certification or recertification of Brucellosis Free Areas.
The principal objective in this study was to develop a more rapid and accurate test for *Vibrio fetus*, especially with mucus from cows or preputial washings from bulls. Contamination has always been a problem, particularly in the preputial washings from bulls. Organisms in many mucus samples were nonviable by the time they were received at the laboratory from the range areas of northern and western Nebraska. Previous experience with other organisms indicated that a direct fluorescent antibody test might overcome most of these handicaps. The diagnosis of vibriosis in bulls using the fluorescent antibody technique has been reported recently.3

MATERIALS AND METHODS

Rabbits were hyperimmunized with the following live cultures of *Vibrio fetus*: Type I (*V. fetus* var. *venerealis*), Subtype I, Type II (*V. fetus* var. *intestinalis*), antigen strain from the National Animal Disease Laboratory (NADL), V-912 from our laboratory, and a strain of *V. fetus* var. *venerealis* from Colorado (designated T-strain). The various strains were grown on cystine heart agar (DIFCO) plus 10 percent whole calf blood under five to ten percent carbon dioxide. After incubation at 37 C. for five days, the growth was harvested in 0.01 molar (M) phosphate-buffered saline (PBS) pH 7.2. The concentration of organisms from each culture was standardized photometrically at approximately three billion cells/ml., and frozen until used. The rabbits were injected via ear vein two times per week, according to the following schedule: initial dosage of 0.25 ml/rabbit, 0.75 ml. for the second, 1.5 ml. for the third, and 2.5 ml. for the fourth, fifth, and sixth injections. The rabbits were bled by cardiocentesis seven days after the last injection, and the serum was collected and frozen.

The gamma globulins from 10 ml. of each antiserum were precipitated with a final concentration of 1.54 M. solution of ammonium sulfate, collected by centrifugation, redissolved in distilled water, and precipitated twice more. The solutions of globulins were then treated in a manner similar to the method of Stair, *et al.*,6 Ammonium sulfate was removed from the globulins by dialysis against several changes of 0.9 percent

*From the Department of Veterinary Science, University of Nebraska, Lincoln.

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sodium chloride. The gamma globulin was diluted to two percent protein with 0.9 percent NaCl, and crystalline fluorescein isothiocyanate (FITC).* dissolved in 0.5 M. sodium carbonate pH 9.0, was added dropwise to the globulin solution to give a final concentration of 20 mg. FITC/Gm. protein and 0.05 M. sodium carbonate. This solution was refrigerated overnight and the following morning the pH was adjusted to 7.5 with 0.5 M. NaH$_2$PO$_4$ and approximately three grams of AG2X4 anion exchange resin (Bio-Rad Laboratories, Richmond, Cal.) was added to the mixture. The resin was separated after a few minutes, and the fluorescein labelled gamma globulin (conjugate) was dialyzed against 0.01 M. sodium phosphate buffer, pH 7.5 containing 15 Gm. AG2X4.

Each conjugate was adsorbed to a column of Type 20 diethylaminoethyl cellulose (DEAE-cellulose)** and eluted by an increasing concentration of NaCl to one molar in 0.01 M. phosphate buffer, pH 7.5. The optical density of each fraction was determined at 495 m$\mu$ for fluorescein and at 280 m$\mu$ for protein and fluorescein. Ratios of the O.D. at 495 m$\mu$ to the O.D. at 280 m$\mu$ were calculated for each fraction. Fractions with a ratio between 0.6 and 1.0 were combined, which corresponded to approximately three to four mg of fluorescein/Gm. protein. For testing the conjugates, aliquots of each were diluted in saline buffered with 0.1 M. phosphate, pH 8.0 containing 1:10,000 Merthiolate (Eli Lilly & Co., Indianapolis, Ind.). Conjugates were tested at 0.25, 0.50, and one mg protein/ml. The diluted conjugate was dispensed with a syringe, fitted with Swinney filter using a 0.45 $\mu$ Type HA cellulose ester membrane (Millipore). Each conjugate was tested against its homologous strain of *V. fetus* and then against other strains. In addition, conjugates were tested for specificity by attempting to stain common contaminating bacteria. The best conjugates for Type I and II *V. fetus* were used to stain field specimens which were submitted to the Veterinary Diagnostic Laboratory.

Three smears of each field specimen, such as stomach contents or cervical mucus, were made on glass slides. The field specimens were also cultured in thiol broth (DIFCO) and on cystine heart agar with five percent blood, 1/40,000 brilliant green and 300 units Mycostatin/ml.² Cultures were examined after five days incubation at 37 C. under five percent carbon dioxide and 95 percent nitrogen, and smears of the growth were made on duplicate slides. Smears of field specimens or the cultures were air-dried and fixed in cold acetone for 10 minutes or longer in a -20 C. freezer. Each of three fixed areas on the slide of the field specimen was encircled with an indelible fast drying marker (Sanford's), then one area was covered with several drops (two or three) of Type I conjugate, a second area with Type II conjugate, and the third was left as an unstained control to detect autofluorescing particles. Duplicate slides containing smears from the cultures were also marked to separate the smears, but one of the duplicates was covered with only Type I conjugate, while the other was covered with only Type II conjugate. All slides were incubated

* Baltimore Biological Laboratories, Baltimore, Md.
** Carl Schleicher and Schuell Co., Keene, N.H.
for 30 minutes in a moist chamber at 37 C. The slides were washed individually on a staining rack using a wash bottle, with three changes of PBS for a total of 10 minutes, then allowed to air-dry. A mounting medium, consisting of nine parts glycerine to one part of 1/15 M. phosphate buffer in saline pH 8.5, was used beneath a No. 1-1/2 coverslip. A drop of Harleco Fluorescence Mountant (Geo. T. Walker & Co., Minneapolis, Minn.) at the corners of the coverslips was used to hold them in place. Slides were either examined immediately, or were refrigerated until the following day. A Leitz ultraviolet (UV) microscope equipped with an Osram HBO-200 high pressure mercury bulb, the 4 mm BG-12 blue light exciter filter, the 2.5 mm OG-1 Blau-abs. (orange) barrier filter and darkfield condenser at a magnification of 500 diameters was used. It was sometimes helpful to search the slides with the Streuscheibe (heat filter) out to allow more UV light transmission. However, a positive diagnosis was not made unless the organisms could be distinguished with the heat filter in place. Pure cultures of V. fetus were typed according to catalase-production, hydrogen-sulfide, production and glycine tolerance using the techniques of Plastridge, et al.4 except that thioglycollate broth (DIFCO) was substituted for thiol medium.

RESULTS AND DISCUSSION

The conjugates for all strains of V. fetus employed were capable of staining homologous cells at a concentration of one mg protein/ml. Conjugates for Types I and II were the most satisfactory for staining all six strains. These were used at a concentration of 0.25 mg. protein/ml. for Type I and 0.5 mg. protein/ml. for Type II. However, fluorescence of homologous cells was still somewhat brighter for each of the conjugates. Conjugates for both Types I and II stained a third strain of V. fetus var. venerealis from New York and three strains of V. fetus var. intestinalis (one from New York and two from Nebraska). Two catalase-negative Vibrio species did not fluoresce with the exception of one which had some questionable fluorescence with Type II. None of the conjugates stained common contaminating bacteria including E. coli, staphylococci, streptococci, gram-positive spore formers (Bacillus spp.), Paracolon spp., or Proteus spp.

During 1965, six pure cultures of V. fetus which were isolated from field cases at the Veterinary Diagnostic Laboratory were tested by the fluorescent antibody (FA) test and all were positive. For the first half of 1966 a total of 41 diagnostic specimens were positive for V. fetus by the FA test, and an additional two by gram stain, for a total of 45 percent of the samples (See Table I). The vast majority of these specimens were aborted bovine fetuses, of which 34 percent were positive, similar to the previous two years. However, 55 percent of bovine cervical mucus samples were positive, in contrast to the previous years in which cultural techniques alone detected V. fetus in only eight percent of the samples. Vibrio fetus was isolated in pure culture from only five bovine fetuses and one ovine fetus; biochemically these were V. fetus var. venerealis and V. fetus var. intestinalis, respectively.
TABLE I
Examinations for Vibriosis by Nebraska Veterinary Diagnostic Laboratory

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<thead>
<tr>
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<td>Aborted fetuses:</td>
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<tr>
<td>Bovine</td>
<td>14/33**</td>
<td>7/49</td>
<td>23/67</td>
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<td>Ovine</td>
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<td></td>
<td>10/13</td>
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<td>Cervical mucus:</td>
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<tr>
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<td>6/11</td>
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<tr>
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<td>0/11</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>Semen—</td>
<td></td>
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<td>1/1</td>
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<tr>
<td>Bovine</td>
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<td></td>
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</tr>
<tr>
<td>Totals</td>
<td>16/59</td>
<td>9/91</td>
<td>43/95***</td>
</tr>
<tr>
<td>Percent positive</td>
<td>27</td>
<td>10</td>
<td>45</td>
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</table>

*Diagnosis made by cultural methods.
**Numerator is the number of specimens positive for *Vibrio fetus*; denominator is the total number of specimens examined.
***Positive diagnosis made by FA in all cases except two.

The five pure cultures of bovine origin all fluoresced strongly with Type I conjugate, but weakly with Type II conjugate. However, the ovine culture appeared to fluoresce equally well with both conjugates. In general, the *V. fetus* organisms usually fluoresced with both conjugates, but the fluorescence was often brighter with one than the other. Approximately two-thirds of the positive bovine specimens fluoresced brighter to the Type I than to the Type II conjugate, while the specimens from sheep usually fluoresced about equally well with both types. However, five positive bovine specimens fluoresced only with Type II conjugate. *Vibrio fetus* var. *intestinalis* may have caused the death of at least three of these cases since the calves were weak or dead at full term. Thus, it may be possible to employ FA tests as a serological procedure to aid in typing various strains of *V. fetus*. Antigenic differences were also noted among various strains of *V. fetus* by Ristic and Murty.5

It was found that dead *V. fetus* cells still fluoresced in dried smears after several weeks of refrigeration, and the cultures were all negative in 12 samples in which *V. fetus* cells were observed in the direct smears. Freezing and thawing *V. fetus* cells before fixing the acetone lysed the cells and reduced the fluorescence, although this was less of a problem with field specimens than with pure cultures of *V. fetus*. Relatively few samples were free of other contaminating bacteria, but apparently the *V. fetus* multiplied even though the former were present. In a number of field cases only the cultures were positive, possibly because the number of organisms were too few to detect in the direct smear. Since contaminating bacteria did not fluoresce, fluorescing *V. fetus* organisms were readily observed. However, some molds autofluoresced under UV light,
Figure 1. Photomicrograph of pure culture of *Vibrio fetus* var. *venerealis* stained with conjugate for Type I *V. fetus*. Note the presence of long chains.

Figure 2. Photomicrograph of *V. fetus* var. *venerealis* organisms in a direct smear of the stomach contents of an aborted bovine fetus, stained with conjugate for Type I *V. fetus*. Typical S-shaped rods are clearly visible along the edge of the smear, as well as mixed with cellular debris. Inset shows a long wavy rod.
making it necessary to examine the untreated area on the direct smears and the cultures for their presence. Since neither the FA tests nor the cultural methods of diagnosis were adequate alone, using both methods together resulted in a more accurate diagnosis.

Mellick, et al.\(^3\) used conjugate prepared for \(V.\) \(fetus\) var. \(venerealis\) to detect vibriosis in the preputial exudate of bulls. His procedure required filtration and high-speed centrifugation prior to examination. Our experience indicates that this may be necessary for preputial samples. In this study, however, most of the cervical mucus samples and fetal stomach contents could be smeared directly on a slide without prior treatment.

**SUMMARY**

Fluorescent antibody (FA) tests aided in the identification of \(Vibrio fetus\) in 45 percent of the aborted fetuses and samples of mucus from cows and bulls which were submitted to the Veterinary Diagnostic Laboratory during the first half of 1966. The percent of the cervical mucus samples which were positive (55 percent) was considerably higher than in the previous two years (eight percent) in which only cultural methods were used. The FA test gave positive results in many cases in which the \(V.\) \(fetus\) organisms were no longer viable, or where other bacteria were also present. The use of conjugates against both \(V.\) \(fetus\) var. \(venerealis\) and \(V.\) \(fetus\) var. \(intestinalis\) was more advantageous than only one in identification of organisms, since certain variety differences were observed by the FA test.

**REFERENCES**


**ADDENDUM**

A disease characterized by fever, diarrhea, salivation and erosions of the buccal mucous membranes was described by Olafson and named bovine virus diarrhea (BVD). Initially he observed rapid spread, with many animals clinically ill and a few deaths, while in later studies, only sporadic fatal cases were noted. Few clinical cases have been observed within herds recently, and most of these died.

Figure 1. The seasonal distribution of Bovine Virus Diarrhea cases recorded in the Large Animal Clinic and post mortem room at the New York State Veterinary College during the years of 1961-1965.

*From the Veterinary Virus Research Institute and the Department of Large Animal Medicine, Obstetrics and Surgery, New York State Veterinary College, Cornell University, Ithaca, New York.

**Professor of Biological Statistics, Biometrics Unit, New York State College of Agriculture, Cornell University, Ithaca, New York.

This work was supported partially by the Spencer T. and Ann M. Olin Foundation, Agway, Inc., the Dairymen's League Cooperative Association, Inc., and other farm organizations and individuals.
Control of BVD in dairy herds requires knowledge about the nature of the disease, its immunity, and the population at risk. Such data were gathered in studies on New York dairy farms.

DESCRIPTIVE EPIDEMIOLOGY OF BVD 1961-1965

Cases of BVD observed at the New York State Veterinary College during the years 1961 to 1965 appeared in every month of the year, with the greatest occurrence in February, March and April (Figure 1). Most cases appeared in cattle under one year of age (Figure 2). Seventy-one percent of cases occurred in cattle six months to two years of age. The interval between appearance of new cases in a herd was three days to two months in cattle housed or pastured together. Clinical signs were observed for one to 21 days before death (average six days). No recoveries were observed in 32 cases. These data may have been biased because only cattle with severe prolonged illness were referred.

SEROLOGICAL INDICATORS OF BVD INCIDENCE

Serological surveys\(^3,5,6\) have shown that 20 to 80 percent of cattle with no history of BVD had evidence of previous infection. This suggested frequent undiagnosed infection. Cattle which failed to develop BVD antibody after BVD infection have been reported.\(^2\) Nevertheless, most

![Bar chart](image_url)

Figure 2. The age distribution of Bovine Virus Diarrhea cases recorded in the Large Animal Clinic and post mortem room at the New York State Veterinary College during the years 1961-1965.
infected cattle did develop antibody and with a sequential testing scheme it was shown that BVD antibody indicated immunity to BVD virus. Virus neutralization tests for the presence of BVD antibody in bovine serums were reliable when standard procedures were used. These data have permitted the conclusion that cattle with antibody have been immunized.

DURATION OF IMMUNITY IN INDIVIDUAL CATTLE FOLLOWING BVD INFECTION

In 1960 the average within-herd BVD antibody prevalence in New York State was estimated as 53 percent. This estimate could not be interpreted meaningfully because the average time of BVD antibody persistence was unknown. Since then, studies have shown that only three (0.6 percent) of 545 cattle actively immunized by natural exposure or by vaccination lost BVD antibody during a three year observation period. The appearance of BVD serum antibody in cattle exposed by contact suggested that reinfection may have been a factor in maintenance of titer. Where such reinfection was not considered a factor, 32 naturally infected cattle maintained titers during a test period of one to three years. Neutralization tests on serums collected from ten cattle over the three year observation period showed minimal loss of titer (Figure 3). A least squares regression line calculated to fit these data indicated the average serum titers declined at the rate of 0.00037 log units per day or about 0.3 log units per 1000 day. By solving the regression equation, the time required for disappearance of titers was determined to be beyond the life expectancy of dairy cattle. (The x intercept was 21.33 years.)

Figure 3. Decline in the average BVD serum titer of ten cows in an unvaccinated dairy herd during the three year period from March 1963 to April 1966.
A herd found to be 100 percent immune in March 1963 declined to 30 percent immune in April 1966 (Figure 4). The serum titers of individual cattle remained relatively constant. This decline in percent of immune cattle within the herd resulted from sale of immune cattle and addition of susceptible cattle. The susceptible cattle were recruited from several sources. These included: calves which eventually became susceptible because of loss of colostrally transferred maternal immunity, calves which were susceptible because their dams were not immune and thus had received no BVD antibody in their colostrum, and some purchased adult replacements which by chance were susceptible. Based on this decline in herd-immunity status, a regression line predicted (Figure 5) that in July 1967 this herd would be composed entirely of individuals susceptible to BVD. Such a prediction was considered speculative because rate of decline in herd immunity was dependent upon the rate at which immune cattle were removed and the decline could be reversed suddenly by reinfection.

THE FATE OF MATERNALLY TRANSFERRED BVD ANTIBODY

Immune cattle consistently transferred immunity to their calves through colostrum. Beginning at birth, serum samples collected from
nine calves retained antibody for an average of 9.23 months afterwards (Figure 6). In another study, serums collected weekly from triplet calves, from a dam that had been immunized by vaccination, lost BVD serum antibody before six months of age (Figure 7). Comparison of these
Figure 7. The least squares regression line of the decline of maternal BVD antibody in triplet calves from a dam vaccinated with combined vaccine one year previously.

figures indicated that rates of decline of maternal immunity in calves from vaccinated dams and from naturally immune dams were similar. The shorter duration of maternal antibody in the calves from vaccinated dams was the result of lower initial titers.

ANTIBODY PREVALENCE AND RATE OF DECLINE IN HERD IMMUNITY PROVIDE AN ESTIMATE OF RECURRENCE TIME

Serological surveys showed antibody prevalence of greater than 50 percent in New York cattle. Herds providing this estimate probably ranged from zero to 100 percent immune. A statement of 50 percent antibody prevalence within herds, along with the estimates of duration of immunity in individuals and the average rate of removal of immune animals from herds, provided an estimate of the average interval with which BVD infection occurred in dairy herds (i.e., the average recurrence time).

A conceptual model was constructed for a hypothetical herd in which the average percent immune through time is 50 percent (Figure 8).

The assumptions implicit in such a model were: First, upon exposure of a dairy herd to BVD virus, all individuals within the herd became infected within a relatively short time so that the herd immunity level was forced to 100 percent. Secondly, this model assumed that infection recurred immediately upon attainment of zero percent herd immunity. Furthermore, this model implied that for every herd reaching zero percent immune another must go to 100 percent immune. Under such conditions it was easy to appreciate intuitively that if the entire population had an average herd antibody level of 50 percent, then the average recurrence
time was five years or less. It also followed that a population with prevalence of antibody in excess of 50 percent must have a correspondingly smaller mean recurrence time.

The above model was considered unrealistic. Experience indicated that frequency of infection was an irregular phenomenon. For a 50 percent antibody prevalence, all herds must be reinfected immediately upon return to zero but if some herds were reinfected prior to return to zero, then others must be reinfected after return to zero.

This more acceptable model of the steady decline and irregular resurgence of BVD immunity within dairy herds is presented in Figure 9.

Varying recurrence times between herds and between subsequent infections within herds have been presented. As in the regular model, maintenance of 50 percent within-herd immunity required that average
recurrence was at least every five years. Likewise, in populations with an average within-herd antibody prevalence greater or less than 50 per-cent, the average recurrence time was respectively shorter or longer. The recurrence time, therefore, was considered an important parameter for making decisions regarding the need for control programs.

THE EPIDEMIOLOGICAL PATTERN

The foregoing presentation described the interactions of BVD virus with the cattle population around Ithaca, New York. Only a small proportion (no estimate available) of those infected developed diagnosed illness. Those that did develop observable clinical signs were from six to 24 months of age, and most of these died. The majority became infected but did not develop diagnosed illness. They survived, retained their immunity, and passed it in colostrum to their offspring, who retained this maternal immunity for an average of six to nine months after birth. Because herd immunity varied from zero to 100 percent, calves with maternal antibody were raised with other calves which had been susceptible from birth because their dams lacked antibody.

CONSIDERATIONS FOR CONTROL OF BVD

It was concluded that control of BVD would require interference with the epidemiological pattern. Vaccine has been made available, but its most effective use required selection of a logical age for vaccination.

As with other species of animals for which colostral studies have been made, colostrally-transferred passive immunity interfered with immunization procedures. This immunity lasted for an average of nine months. Calves were found to attain susceptibility (which implies a concurrent amenability to vaccination) at different ages, because each received a different initial complement of antibody from its own dam, while some received none at all. Those with higher titers reached susceptibility at a later date.

If the practice of individual prevaccination testing of calves for susceptibility and frequent revaccination were excluded as economically unfeasible, a single age must be sought to assure successful vaccination of the greatest number of calves at the earliest possible age. If the antibody prevalence in the parent population was less than 10 percent the decision would be easy, because most newborn calves would be susceptible and could be vaccinated successfully. Early vaccination would be indicated because less than 10 percent would fail to become immunized. Also, if the antibody prevalence within the parent population was greater than 90 percent the decision would be easy, because more than 90 percent of calves would have maternal antibody and vaccination could be postponed. The actual situation had 50 percent antibody prevalence in the parent population. A rational decision was made difficult because early vaccination would result in a 50:50 chance of failure and late vaccination would result in a 50:50 chance of permitting calves to remain susceptible during early calfhood.
SUMMARY

The duration of active BVD immunity is estimated to extend well beyond the life expectation of a dairy cow. A dairy herd, once infected, will gradually revert to a state of susceptibility unless reinfection occurs. The rate at which the herd becomes susceptible is related only to the rate of removal of immune cattle and their replacement by susceptible cattle. If the average within-herd prevalence of BVD antibody remains above 50 percent and if the average dairy herd turns over completely in five years, then each herd in the state must be infected on the average at least once every five years, and in populations with a higher antibody prevalence the recurrence time is correspondingly shorter. When the prevalence of antibody in a parent population, the duration of immunity in individual animals, and the rate at which immune animals leave the herd are known, it is possible to estimate a maximum average recurrence time. These parameters are necessary for making decisions in any disease control program.

REFERENCES

Throughout the United States, many scientists are trying to solve disease problems in neonatal calves. Because the enteritis (scours) syndrome is probably the most serious of these diseases, and causes the greatest losses, most of the research is directed at this condition. This report is issued so that all interested individuals and organizations can be informed of the research now underway. If there are oversights or omissions, they should be brought to the attention of Dr. Norman L. Garlick, Chief Staff Veterinarian, Viral and Parasitic Cattle Diseases, Animal Health Division, ARS, USDA, Federal Center Building, Hyattsville, Maryland 20782. Supplements to this report will be issued as additional information becomes available.

**Cooperative State Research Service, USDA:**

Experiment Stations throughout the United States are directing considerable effort toward controlling this important, costly disease problem (enteric disease of neonatal calves). Ten Stations in the West are cooperating, coordinating their research under Western Regional Research Project W-88, Enteric Disease of Neonatal Calves. The principal objectives of this and other groups are to determine the clinical signs and disease manifestations, to investigate the causes and how each affects the animal, and to develop effective control procedures. The effect of microorganisms, hormones, enzymes, environment, nutrition, toxins, body defense mechanisms and physiology are being explored. These Stations, combined, anticipate using about a quarter of a million dollars in this effort during 1966. The reports of the individual Stations will appear under the names of the States in which they are located.

**Animal Disease and Parasite Research Division, ARS, USDA:**

Investigations on "scours" of neonatal calves are being conducted at the National Animal Disease Laboratory as part of the research on enteric diseases of cattle. Major emphases are on the transmission and experimental production of the bovine viral diarrhea-mucosal disease complex. One phase of this research is to determine the susceptibility of the neonatal calf to the virus of bovine viral diarrhea. Another objective is to determine the role of the neonatal calf and its dam in the transmission of this disease within the herd. A third goal is to clarify the relationship of "immunologic tolerance" and the mucosal disease syndrome where the virus of bovine viral diarrhea persists but some affected animals do not develop antibodies against it.

The Division also has a research contract with the University of Idaho to conduct investigations on resistance to enteritis (scours) among calves.
RESEARCH ON DISEASES OF NEONATAL CALVES

Department of Animal Pathology, College of Agriculture and Agricultural Experiment Station, University of Arizona:

The Department of Animal Pathology is participating in the Regional Research Project W-88. In addition, a State project on the causes of mortality of young calves has been in progress for about six years. Dr. Ned Rokey, working at the Mesa Station, has concentrated on identification of the disease-causing microorganisms associated with the death of calves, the lesions produced, diagnostic methods and control measures. Included in control measures is the experimental evaluation of vaccines, serums and drugs (biologicals and chemotherapeutic agents). In the course of his work, Doctor Rokey has contributed significantly toward putting the role of the various salmonella bacteria in perspective.

At Tucson, work is being done on the cause and course of the disease in dairy calves. Also, fetal hydrocephalus (abnormal accumulation of fluid in the brain cavity) is being studied. The resistance (or immune status) of the dam and newborn calf in relation to the development of fatal calfhood enteritis has also been a subject of intense interest.

Division of Agricultural Sciences, Agricultural Experiment Station, University of California:

The Experiment Station is cooperating in the Regional Research Project W-88. The incidence and causes of enteric diseases in dairy and beef herds is being studied through organized calfhood health programs. The blood of affected calves is being examined, as well as that of normal calves, to find out what changes take place during disease. Bacteremia (septicemia) caused by Escherichia coli (E. coli) and other similar organisms in colostrum deprived calves is being studied. The relationship between age and clinical signs will be determined, particularly as they relate to changes in the blood. "Germ-free" calves will be reared for one-month periods and studied extensively. These calves will be delivered by cesarean operation and held in germ-free environments. The effect of E. coli on these calves will be compared to that on similar calves raised under other conditions.

Field trials with E. coli bacterin from a commercial source were carried out during 1965. Cows were given two injections 30 to 60 days prior to parturition. The offspring of vaccinated and nonvaccinated cows had an equal incidence of scours. Also, vitamins A, D, and E, along with an experimental drug, were studied for their possible protection against calfhood diseases.

Other trials for the future are to evaluate commercial serums given both by injection and by mouth, and injectable biologics which contain E. coli endotoxins.

Colorado State University:

Colorado State University is participating in the Regional Research Project W-88. The objective of the Colorado group is to study the role of viruses in neonatal calves and to determine the characteristics of viruses.
that are isolated from calves with enteric diseases. This phase is being done by Dr. J. Storz. Using the viruses isolated by Doctor Storz, Drs. R. W. Phillips and K. L. Knox are studying the water balance in affected calves, and the effect of enteric diseases on the rate of metabolism in neonatal calves. In addition, the transmission of the isolated viruses to susceptible calves is being investigated.

Veterinary Science Department, University of Florida:

The Veterinary Science Department is carrying out a study of salmonella organisms, including field investigations and the evaluation of various drugs used to treat animals affected by these bacteria.

School of Veterinary Medicine, University of Georgia:

The School is interested in doing research on the effects of vitamin A deficiency in young dairy calves. This work will depend upon finding a source of the necessary research funds.

State of Hawaii:

The State of Hawaii reports that "calf scours" has not been troublesome in that region. However, field investigations and supporting laboratory work are done on request. Results of these investigations are published for the benefit of others.

Department of Veterinary Science, University of Idaho:

Work continues on identification of agents causing disease in neonatal calves, including bacteria, viruses, combinations of these, and possible additional unknown factors. The possibility of infection prior to birth has also received consideration. A new project is designed to investigate the resistance of neonatal calves to enteritis, including the role of gamma globulins and other blood serum proteins and specific antibodies. Preliminary work has been accomplished including obtaining the necessary equipment, standardizing test procedures, acquiring and training personnel, locating suitable experimental animals and preparing facilities. The Veterinary Research Laboratory at Caldwell is participating.

College of Veterinary Medicine, University of Illinois:

The College of Veterinary Medicine is investigating leptospirosis in calves under the leadership of Dr. L. E. Hanson. Further projects include studies of this disease in pregnant cows. *Leptospira grippotyphosa* and *L. hardjo* have been associated with abortions, still-births, retained placentas, and calves weak at birth. Breeding problems have also been reported in animals carrying antibodies for these two organisms. Herds with animals positive to blood tests for these *Leptospira* types are scattered throughout the State, and are present in the deer population.

Purdue University:

Purdue University has just completed a research project on the value of a proprietary drug in the treatment of calf scours. A report of the study will probably be available in the near future.
Kansas State University:

Dr. Embert Coles reports that work is underway on salmonellosis in calves. A survey on salmonellosis has been proposed by the Experiment Station to determine its incidence, prevalence, presence of carriers, and response of animals to vaccines and serums used in its control.

College of Veterinary Medicine, University of Minnesota:

Research is conducted on selected individual herds when clinical outbreaks of neonatal calf diseases occur. Investigations are designed to fit the individual situation in each herd. Such factors as management, treatment and cause are included. Results of these studies will be made available in the future.

School of Veterinary Medicine, University of Missouri:

Dr. D. E. Rodabaugh, prior to July 1965, conducted a limited investigation on the production of a pneumonia-enteritis syndrome by inoculating young calves with parainfluenza virus and pasteurella organisms. Both \emph{P. multocida} and \emph{P. hemolytica} were tried. Results indicated that both virus and bacteria were needed to produce even mild transitory respiratory disease with some enteritis. The best evidence of disease was produced following the third serial passage of the agents in experimental animals, but the trials were not carried beyond that point. If the work is to be repeated, the investigators believe that colostrum-deprived calves should be used as experimental animals.

Veterinary Research Laboratory, Montana State University:

The Veterinary Research Laboratory is cooperating in the Regional Research Project W-88. The work is related to the cause of the disease, but details have not yet been made available.

Montana Livestock Sanitary Board Diagnostic Laboratory:

The Diagnostic Laboratory has been conducting investigations on the cause of outbreaks of "scours" in calves. All calves submitted to the Laboratory which are affected with, or have died from, "scours," are subjected to an intensive examination including attempts to isolate bacteria which may have caused the disease. The predominant isolations have been \emph{E. coli}. These bacteria are being studied to determine which are the strongest antigenic strains—that is, which stimulate the greatest protective response in inoculated animals. It is hoped that the work will lead to the production of effective vaccines and immune serums. These investigations have been in progress for several years, and an account is to be published soon.

Veterinary Science Department, University of Nebraska:

Major outbreaks of "calf scours" are being investigated at the request of cattle owners and their veterinarians. The Department hopes to initiate a research project on this problem and has taken steps in that direction. The Department has also carried out intensive observations on a
hydrocephalus syndrome in newborn calves which is called hereditary encephalomyopathy. Plans are underway to continue this work in the future if research funds become available.

Animal Science Department, University of Nevada:

The Department is cooperating in the Western Regional Project W-88. Clinical descriptions, tissue damage and herd history will be recorded in connection with outbreaks and correlated with laboratory findings. The cause of each outbreak will be determined, if possible, through the culture of specimens for bacteria, and the isolations of viruses in tissue culture. Antibody levels to certain selected viruses will be determined (infectious bovine rhinotracheitis, bovine viral diarrhea, and parainfluenza 3). Suitable specimens will also be checked for epizootic bovine abortion. If isolations are successful, attempts will be made to reproduce the diseases by inoculation of pregnant cows and suitable calves. If inoculations result in disease, experimental vaccines will be produced and tested. In addition, selected biologics available from commercial sources will be evaluated in relation to diseases of neonatal calves.

Department of Veterinary Science, North Dakota, State University:

A project is now in progress to determine normal limits of various blood and serum constituents in young calves offered for public auction. Blood samples are collected at the markets from calves under two weeks of age. It is hoped that some criteria may be established which will permit early prediction of disease resistance (or lack of it) in neonatal calves. The auction markets furnish the names and addresses of the purchasers of the calves, and questionnaires are mailed to them two weeks after purchase to find out whether or not the calves have been ill. Death rates for calves thus handled will be determined for the various seasons. Subsequent studies will be directed toward evaluating procedures of prevention and treatment of diseases in neonatal and very young calves.

Department of Veterinary Medicine, Oregon State University:

Dr. D. E. Mattson is leading a project on neonatal calf losses as a part of the Western Regional Project W-88. Comprehensive investigations of outbreaks are conducted in beef herds where severe dysentery and mortality have been troublesome in the past. Suitable specimens from affected animals are examined for disease-causing bacteria and viruses. Any organisms recovered are to be identified and characterized, and their disease-causing ability determined. Various biologics are being evaluated, some commercially produced and others specially prepared, both independently and in cooperation with other research units and investigators working on the problem of disease in neonatal calves in Oregon and adjacent areas. A solution to the problem of neonatal calf losses is urgently being sought.
RESEARCH ON DISEASES OF NEONATAL CALVES

Veterinary Division, Department of Agriculture, State of Oregon:

The Department has a number of cooperative projects underway related to the diseases of young calves. One involves the injection or the oral administration of a commercially available antibacterial serum to calves in herds where calf enteritis has been a problem. Another trial includes the administration of large doses of serum derived from cows hyperimunized with *E. coli* bacterins and the bacteria causing enterotoxemia. Both of these projects are designed to test the potential protective properties of the serums.

In another trial, bacterins have been prepared from selected organisms harvested during previous outbreaks of enteritis in Oregon. These are administered to cows in two injections 30 to 60 days prior to calving. Late calvers receive a third injection. The results of the 1966 trials are now being summarized.

In an attempt to provide protection to newborn calves in herds which have had severe outbreaks of "scours" in the past, a colostrum bank was established in Baker County. The colostrum of dairy cows from the first two milkings was placed in one-quart containers and frozen for future use. Participating cattlemen could draw on the bank as their calves were dropped. Evaluation of this project is proceeding.

These projects have been variously carried out with the cooperation of the Animal Health Division, ARS, USDA; the Diagnostic Laboratory, Oregon State University; a commercial laboratory which produced the bacterin; and Dr. F. W. Frank, University of Idaho Branch Experiment Station, Caldwell, Idaho.

Department of Veterinary Science, College of Agriculture, The Pennsylvania State University:

Under the leadership of Dr. P. J. Glantz, much has been and is being accomplished in the field of neonatal calf diseases. All of the known *E. coli* antiserums against *O, K and H* antigens, as well as all reciprocally cross-absorbed antiserums are being used to completely identify the *E. coli* strains isolated from any source. Serotyping has been done for numerous research workers throughout the United States.

Evidence has been obtained that certain serotypes of *E. coli* are more frequently isolated from diseased than normal animals; a marked difference in pathogenicity (disease-causing ability) has been demonstrated. The role of *E. coli* in both experimentally induced infections and natural outbreaks in the field has been studied. These experiments have demonstrated the importance of early ingestion of colostrum in preventing or alleviating disease caused by *E. coli*. A limited field study on the use of colostral whey to protect calves has also been done, but the results were inconclusive.

The work will be continued and expanded, depending upon available financial support. In this connection, a reference center for *E. coli* associated with animal disease has been established at the Department. This center will serve North America as the only laboratory for complete
serotyping of \textit{E. coli} isolates, a very important and indispensable service. The reference center is to be self-sustaining; that is, a charge will be made for serotyping, for cultures, and for serum. This center could serve as the source of most valuable information on these diseases and for cooperative research with other scientists.

The Department's activities have not been limited to calves, but have included swine, sheep, rabbits, poultry and other species. A data retrieval system is urgently needed to correlate the information on hand and make it available to others.

\textit{The School of Veterinary Medicine, University of Pennsylvania:}

Dr. A. M. Merritt has been studying neonatal diarrhea with particular emphasis on disturbances in metabolism.

\textit{Veterinary Science Department, Utah Agricultural Experiment Station, Utah State University:}

The Veterinary Science Department is cooperating in the Western Regional Project W-88. Calves dying within a few days of birth have been examined, with full laboratory support, and a number of disease-producing microorganisms have been isolated from one or more of the calves. Included were coliform bacteria, proteus species, streptococcus species, pseudomonas species, \textit{Salmonella dublin}, and \textit{S. typhimurium}. Also, a virus was isolated from the intestinal tract of calves from one herd troubled with pneumoenteritis. Future work is to continue along the same lines, isolating and identifying disease-causing organisms and testing their ability to produce disease in experimental animals.

\textit{Department of Veterinary Science, College of Agriculture, Virginia Polytechnic Institute:}

The Department of Veterinary Science has in progress a project on the pathology and pathogenesis of \textit{E. coli} diseases in the neonatal and young calf, but details are not yet available.

\textit{Department of Veterinary Microbiology, Washington State University:}

The Department of Veterinary Microbiology is cooperating in the Western Regional Project W-88. Under D. T. Moll, the work was started in July 1963. Most of the activity so far has been in establishing experimental procedures. The long-range objectives are to define the causes and pathogenesis of enteric diseases of neonatal calves with special studies of the environment and the endocrine glands. Both "normal" and "stress" conditions will be explored. The relationship of abnormal adrenal gland function to the absorption of globulins (antibody-containing proteins) from the intestinal tract will be studied in detail. In connection with absorption of globulins, a simple field test has been developed, and is being evaluated, to quickly determine such absorption in newborn calves.

Newborn calves are to be exposed to varied environmental temperatures in a controlled temperature chamber. Blood samples will be taken at intervals and examined for corticosteroid (hormones from the adrenal
gland) and gamma globulin content. These will be compared with samples from other calves maintained at room temperature, and with samples taken from normal and sick calves in the field. Calves will also be given corticosteroids immediately after birth, and their ability to absorb colostral gamma globulins from the intestinal tract will be compared to normal untreated calves.

Division of Veterinary Science, University of Wyoming, and the Wyoming State Veterinary Diagnostic Laboratory:

Cooperative investigations into losses of neonatal calves in Wyoming are in the beginning stages. Ranchers and veterinarians are being contacted to ask them to get in touch with research personnel when clinical outbreaks occur. This is a part of a larger project which includes infections of the reproductive tract, abortions and calf losses. A limited number of herds will be selected so that long-range observations can be made. Suitable materials will be collected for attempted isolation of microorganisms. Serum samples from sick cows and calves will be examined. Organisms isolated from cows and calves will be studied, identified, and, where indicated, will be inoculated into newborn calves. Cows in late gestation (pregnancy) will be inoculated with agents isolated from calves to determine any possible effects on the calves subsequently born to mothers infected with microorganisms associated with diseases of young calves. Several microorganisms have been isolated from the intestines of young calves suffering from "scours." These are being investigated to determine their possible participation in death losses.
Reports from various parts of the country in the past ten years attest to the increasing importance of the bovine encephalitides. Retrieval of data from the records of the Iowa Veterinary Diagnostic Laboratory reveals that from August 1, 1964 to August 1, 1965, 164 of the 546 bovine diagnoses made were encephalitides (Table I).

**TABLE I**

| Bovine Encephalitides Diagnoses at the IVDL From August 1, 1965 to August 1, 1966 |
|---------------------------------|-----------------|
| Infectious Thrombo-Embolic Meningo-Encephalitis | 55 |
| Polio | 50 |
| Listeriosis | 25 |
| Rabies | 28 |
| Unclassified Encephalitis | 6 |
| **Total** | **164** |

The clinical signs of these diseases are often so similar as to make a definite clinical diagnosis difficult. A veterinarian should approach the problem of a bovine with central nervous signs in terms of a differential diagnosis including, at least, the first four disease entities shown in Table I. It is with these conditions this discussion will be primarily concerned.

**Infectious Thrombo-Embolic Meningo-Encephalitis**

Infectious thrombo-embolic meningo-encephalitis is an acute, febrile disease of cattle caused by a short gram negative rod and characterized by ataxia, depression and coma. The organism isolated from cattle with this disease by Kansas workers has been classified as *Actinobacillus actinoides*-like, whereas other investigators have regarded the organism as possibly a *Hemophilus sp.*

A febrile response occurs early in the disease. Incoordination, weakness, excitement, prostration, convulsions, and coma may be observed. Gross lesions in the brain consist of reddish-brown foci of necrosis and hemorrhage throughout the brain which result from thrombosed blood vessels. Histopathologic examination of brain sections reveals vasculitis and thrombosis of blood vessels of the brain and meninges. Necrosis, infiltration by polymorpho-nuclear leucocytes and hemorrhage are observed in the tissue surrounding the affected blood.
vessels. The causative organism can usually be isolated from affected brain tissue on blood agar incubated at 37°C under 10 percent carbon dioxide. Generally speaking, a tentative diagnosis can be made if the characteristic gross lesions are present. In many cases, however, the tissue changes are not of such a magnitude to be apparent and histopathologic examination is required.

**Polioencephalomalacia**

Polioencephalomalacia in cattle is considered by most workers to be a non-infectious disease. Some of the early investigators of this condition considered a chronic selenium poisoning as the etiology. Attempts to reproduce the disease by feeding selenium have not yielded positive results. The most prevalent thought today is that this condition is a form of toxicosis. Feed, especially silage, has been incriminated as harboring a toxic agent capable of producing the disease. Attempts to reproduce this condition with infectious and non-infectious agents have consistently been negative.

It is not uncommon to find cattle affected with this condition prostrate and comatose. However, some of the clinical signs observed in this disease have been muscular tremors, twitching of the facial muscles, excessive salivation, impairment of prehension and blindness. The animal with polioencephalomalacia is afebrile.

The lesions are found chiefly in the cerebral cortex and consist of multiple foci of necrosis which appear yellowish and are often sharply demarcated from the normal brain tissue.

Neuronal degeneration is present in the necrotic foci which are limited to the gray matter. Capillary and endothelial proliferation are observed adjacent to the necrotic areas. Also accumulations of compound granular cells occur in and around the areas of necrosis. Perivascular cuffing and meningeal infiltration by lymphocytes are observed in association with the lesion.

Diagnosis of polioencephalomalacia depends on histopathologic examination of brain, since in most cases advanced gross lesions are not present.

**Listeriosis**

Listeriosis is an infectious disease occurring in cattle caused by a bacterium, *Listeria monocytogenes*. It has been felt by many that the occurrence of the disease is related to certain feeds or feeding practices.

Clinical signs of the disease include unilateral facial paralysis, excessive salivation, circling, prostration and coma. Cattle with listeriosis are febrile. There are no typical gross lesions in the brain of cattle with this disease. However, histopathologic examination of brain sections reveals some rather characteristic tissue changes which are usually found in the brain stem. Micro-abscesses consisting of polymorphonuclear leucocytes, lymphocytes and macrophages are scattered throughout the affected tissue. Perivascular cuffing and meningitis are observed of which lymphocytes and macrophages are the primary cellular constituents.
Neurons in close proximity to the micro-abcesses undergo degenerative change.

Recovery of the organism from brain is of course the basis of an etiologic diagnosis. However, it is fortunate in view of the fact that isolation of the organism is sometimes rather difficult, that the histopathologic lesions are characteristic enough to warrant a diagnosis being made even in the absence of positive bacteriologic findings.

**Rabies**

Rabies is a form of encephalitis caused by a virus. It is usually recognized clinically either in the excitement or furious form or the depression or dumb form. Irritability, hyperesthesia, excessive salivation, straining, hoarse or low pitched bellowing, sexual excitement, paralysis and coma are clinical signs that have been observed. Increased body temperatures occur in those affected animals in which a lot of muscular activity has taken place.

Gross lesions are not evident in this disease. Histopathologically, perivascular cuffing, glial nodules are present in the pons, cerebellum and cervical cord. Negri bodies, the intracytoplasmic cellular inclusions of this disease, are often found in the Purkinje cells. The diagnosis of this disease in the field, can be a very difficult one, as most veterinarians are well aware. One-hundred and seventy-three bovine brains were submitted to the Iowa Veterinary Diagnostic Laboratory August 1, 1965-August 1, 1966, requesting rabies examinations. The fluorescent antibody test, and histopathologic study were done on each. The fluorescent antibody test is an excellent tool in the diagnosis of rabies. In addition to this, hostopathologic study of these brain tissues is of great aid in establishing diagnoses in those cases in which the fluorescent antibody test is negative. Of the 145 negative cases, 26 diagnoses of other encephalitides were made (Table II). The diseases discussed here do not comprise the complete differential diagnosis of encephalitis in the bovine. Aujesky's disease, sporadic bovine encephalomyelitis, enterotoxemia, and lead poisoning are but a few diseases that could also be included in this regard.

Cattle infected with the virus of Aujesky's disease often have the characteristic signs of pruritis, self mutilation, and tremors enabling the

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Encephalitis Diagnoses in Rabies Suspects at IVDL August 1, 1964 to August 1, 1965</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections Thrombo-Embolic Meningo-Encephalitis</td>
<td>3</td>
</tr>
<tr>
<td>Polio</td>
<td>9</td>
</tr>
<tr>
<td>Listeria</td>
<td>12</td>
</tr>
<tr>
<td>Unclassified Encephalitis</td>
<td>2</td>
</tr>
<tr>
<td>No lesions of encephalitis</td>
<td>119</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
</tr>
</tbody>
</table>
veterinarian to make a clinical diagnosis. In the absence of these signs the diagnosis can be made by subcutaneous injection of emulsified spinal cord into rabbits. A severe pruritis will develop and the rabbit will severely traumatize the affected area.

Sporadic bovine encephalomyelitis, caused by a virus of the psittacosis-lymphogranuloma group is characterized by weakness and incoordination. Other nervous signs include circling, hyperexcitability, opisthotonus and convulsions. Characteristic post mortem findings of pleuritis, pericarditis and peritonitis will help in making a diagnosis of this disease. Elementary bodies can be observed in the cytoplasm of macrophages in the serosal exudate and brain of affected animals.

Enterotoxemia, caused by a toxin of Clostridium perfringens may manifest the clinical signs of incoordination, prostration and convulsions in cattle. Necropsy examination will often reveal hemorrhages in the serosal membranes. There are usually no gross or microscopic lesions in the brain. Demonstration of the toxin in the intestinal content can be accomplished by the mouse inoculation test.

Cattle with lead poisoning often show such signs as blindness, incoordination, muscle tremor, salivation and anemia. In the more acute form, convulsions, opisthotonus and muscle tremors are observed in the affected animal. There are no gross or microscopic tissue changes that are pathognomonic for this disease. Chemical analysis of bone, liver, kidney and rumen content for lead may offer the only means of a definite diagnosis. It can be said that the initial four diseases considered here do comprise a great percentage of the encephalitis problems of the bovine in many parts of this country today.

REFERENCES

PRECONDITIONING OF FEEDER CATTLE PRIOR TO INTERSTATE SHIPMENT
(A Preliminary Report)

Richard F. Bristol, D.V.M., M.S.*

INTRODUCTION

The findings and recommendations upon which this report is based are the results of a preliminary survey conducted by the Department of Clinical Sciences of the College of Veterinary Medicine at Iowa State University. The survey was undertaken to determine if any correlation might exist between certain management practices of the rancher-source of feeder calves, the practices of the feeder-seller, and the disease problems encountered by the feeder-seller.

Three geographical areas were surveyed for sources of feeder calves. These source areas were the counties surrounding Choteau, Montana, Ainsworth, Nebraska, and Mitchell, South Dakota.

All cattle that were traced to their final destination were shipped to the state of Iowa for their final feeding period.

The number of farms or ranches involved in the survey was 63, averaging 171.9 calves sold per farm. The extremes in numbers of calves marketed for feeding purposes per farm or ranch varied from 50 to 864 animals per year. The total numbers of animals involved in this initial work were 10,832. The total numbers of feeder calves traced to their ultimate destination were 2,656 calves.

The questions asked of the rancher source of the feeder calves were as follows:

1. Number of calves sold
2. Sex
3. Age at date of sale
4. Method of sale
   a) private negotiation or contract
   b) public auction
5. Method of shipment
   a) rail
   b) truck
6. Surgical procedures performed by owner-source
   a) castration
      1. those castrated at least 30 days prior to sale
      2. those castrated less than 30 days prior to sale
   b) spay or other procedures
      1. those spayed at least 30 days prior to the sale
      2. those spayed less than 30 days prior to sale

*Associate Professor, College of Veterinary Medicine, Department of Clinical Sciences, Iowa State University.

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7. Vaccination procedures employed prior to shipment
   a) type of vaccines administered
   b) method of administration
   c) date of administration
      1. at least 30 days prior to selling and shipment
      2. less than 30 days prior to selling and shipment
8. Antibiotics administered prior to shipment
   a) oral administration
   b) parenteral administration
   c) type used
   d) date administered
9. Did any co-mingling with other animals take place at the time of
   sale?
10. Were pesticides administered?
    a) type
    b) when administered
11. When were animals weaned?

The feeder seller was queried in these areas:

1. Date of arrival of the calves
2. Condition of the calves upon arrival
   a) Were any animals visibly ill?
   b) Were veterinary services required?
   c) Veterinarian's diagnosis.
3. Feeding and handling practices at the time of arrival
   a) types of feed available to the calves
   b) availability of water
   c) availability of shelter
4. Medical practices upon arrival
   a) vaccines given
      1. type
      2. how administered
      3. date administered
   b) other chemo-therapeutic agents employed
      1. antibiotics
      2. sulfonamides
      3. route of administration
   c) pesticides used
      1. type
      2. date of administration
      3. method of administration
   d) Were veterinarians employed in the above procedures?
      1. how employed
5. Disease problems after arrival
   a) type of problem
   b) date the animals were first affected
   c) steps taken to eliminate the condition or conditions
1. professional assistance
2. chemo-therapeutic agents employed
3. method of administration
d) morbidity
e) mortality

6. Additional comments of the owner in regard to health and general well being of the animals upon final sale

RESULTS

It is not within the scope of this paper to discuss or report on all of the findings in this survey. This preliminary report will confine itself to certain pre-selling practices of the rancher-seller, shipment of the animals and the method of selling.

1. Method of sale
   (see Chart 1)
2. Vaccination procedures
   (see Chart 2)
3. Weaning procedures
   (see Chart 3)
4. Methods of shipment
   (see Chart 4)
5. Surgical procedures
   (see Chart 5)

Slightly over one-fifth of the animals involved in the survey were traced to their eventual destination. It was found that animals sold by private contract were much easier to trace. The owners were more cooperative in answering questions relative to the animals and more detailed information could be obtained. Of the animals sold at auction, only 710 animals of the total number could be traced; this represented only eight percent of the animals surveyed.

It is from animals sold on private contract that the correlations have been obtained. In evaluating the data obtained, the feeder-sellers estimation on record of the number of cases of respiratory infection that were treated in a particular lot during the first 30 days after arrival on the premises are compared with the management practices of the rancher-seller.

SUMMARY AND DISCUSSION

The weaknesses of a survey of this type are quite obvious. The information received from the rancher-seller and the feeder-seller are dependent on few, if any, written records and the memory of the individual owner. By and large, the records of professional people in regard to exact numbers of animals treated, the types of medication involved, and the response to treatment was woefully unavailable. By the same token, definitive diagnosis of animals affected with an illness during the first 30
### CHART 1

**Method of Sale**

<table>
<thead>
<tr>
<th>Number of Calves</th>
<th>Auction</th>
<th>Percent</th>
<th>Traced</th>
<th>Percent</th>
<th>Private Sale</th>
<th>Percent</th>
<th>Traced</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,832</td>
<td>8,882</td>
<td>82</td>
<td>710</td>
<td>8</td>
<td>1,950</td>
<td>18</td>
<td>1,198</td>
<td>38</td>
</tr>
</tbody>
</table>

*Percent figures are given in the nearest whole number.

### CHART 2

**Vaccination Procedures**

<table>
<thead>
<tr>
<th>Number of Calves</th>
<th>Vaccinated</th>
<th>When Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(At least 30 days prior to sale)</td>
</tr>
<tr>
<td>10,760</td>
<td>Clostridium Chauvei Septicus</td>
<td>8,470</td>
</tr>
<tr>
<td></td>
<td>Pasturella</td>
<td>1,340</td>
</tr>
<tr>
<td></td>
<td>I.B.R.</td>
<td>2,142</td>
</tr>
<tr>
<td></td>
<td>B.V.D.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Leptospira pomona</em></td>
<td></td>
</tr>
</tbody>
</table>

*One owner did not answer this question.

### CHART 3

**Weaning Procedures**

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>(Weaned less than 30 days prior to sale)</th>
<th>(Weaned more than 30 days prior to sale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,264*</td>
<td>2,647</td>
<td>4,617</td>
</tr>
</tbody>
</table>

*Some ranchers did not answer this question.

### CHART 4

**Method of Shipment**

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Method of Shipment</th>
<th>Number of Hours in Transit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Truck</td>
<td>Rail</td>
</tr>
<tr>
<td>9,628*</td>
<td>8,276</td>
<td>1,352</td>
</tr>
</tbody>
</table>

*Some ranchers did not answer this question.

### CHART 5

**Surgical Procedures**

<table>
<thead>
<tr>
<th>Number of Calves</th>
<th>Castrated</th>
<th>Surgery Not Performed</th>
<th>Surgery Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(At least 30 days prior to sale)</td>
<td>(Within 30 days of sale)</td>
</tr>
<tr>
<td>10,832</td>
<td>6,427</td>
<td>4,279</td>
<td>6,076</td>
</tr>
</tbody>
</table>
The Time in Transit Compared to Respiratory Involvement

Animals traced from rancher-seller to feeder-seller time in transit when compared to rate of respiratory disease.

Total number of animals: 2,656
Cases of respiratory involvement: 231 (10)
In those less than 24 hours in transit: 37 (16)
In those over 24 hours in transit: 64 (27)
In those over 36 hours in transit: 130 (56)

*Percent expressed to the nearest whole number.

Incidence of Respiratory Involvement when Compared with Castration Practices

<table>
<thead>
<tr>
<th>Castrated</th>
<th>No. of Calves</th>
<th>No. Castrated</th>
<th>(At least 30 days prior to sale)</th>
<th>(Within 30 days of sale)</th>
<th>Uncastrated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10,832</td>
<td>6,427</td>
<td>6,076</td>
<td>571</td>
<td>4,279</td>
</tr>
<tr>
<td>No. of Calves Traced</td>
<td>2,656</td>
<td>2,227</td>
<td>2,106</td>
<td>121</td>
<td>429</td>
</tr>
<tr>
<td>No. of respiratory condition cases treated</td>
<td>231</td>
<td>192</td>
<td>27</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Percent treated*</td>
<td>10</td>
<td>8</td>
<td>21</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*Percent figures are given to the nearest whole number.

Weaning Practices in Comparison to Respiratory Involvement

<table>
<thead>
<tr>
<th>No. of Calves</th>
<th>Total no. of cases</th>
<th>231</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Weaned 30 days prior to sale)</td>
<td>12</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>(Weaned within 30 days of sale)</td>
<td>219</td>
<td>99.5</td>
<td></td>
</tr>
</tbody>
</table>

days after arrival was extremely difficult to establish again due to inadequate records and lapses of memory. Thus, the term "respiratory conditions" is used. It has been established that respiratory involvement can exist as a secondary condition to other disease states.

None the less, the information that has been received is of some value in establishing a trend of infection rate.
The numbers involved in this survey should be expanded so that these trends can be established and the habits of interstate movement of animals recorded.

It was noted early in the survey that it was extremely difficult to trace the movement of animals sold at public auction. In some cases (11) the animals were traced through three states and then lost. One group of 97 calves was followed through four states, six professional dealers of cattle and finally lost in the Omaha, Nebraska stockyards after having been grouped with cattle from Cherry County, Nebraska. The cattle were originally from Mitchell, South Dakota. The records of the public auction are almost impossible to examine or to trace.

One of the most startling incidents of the entire survey concerned a group of 53 calves sold at public auction at Ainsworth, Nebraska. These animals were traced through four professional dealers, two public auctions, one public stockyard, and a private sale. The time elapsed between the date of the original sale and their final destination was eight days! This group of calves had a 100 percent incidence of a respiratory condition and a total death loss of 48! It would seem that the time of weaning calves is a factor in the incidence of recognizable respiratory infection within thirty days of arrival upon the farm. The effects of vaccination are difficult to determine other than to state that in the small number of animals traced, the use of the Viral Vaccines at the time of shipment when correlated with the incidence of respiratory infection shows a distinct rise in incidence.

The use of the auction barn as a method of sale and acquisition of animals tends to show an increase in the incidence of respiratory infection.

There is a radical increase in correlating the castration practices with the incidence of respiratory infection. However, this is based on a small numerical sampling.

A correlation between the time in transit and the incidence of respiratory disease is well defined and the numerical sampling is adequate. It would seem that as the time in transit increases there is a corresponding rise in the incidence of respiratory infection.

RECOMMENDATIONS

Although this survey is in its preliminary stages and much information is still to be obtained, it would seem that certain practices of disposing of feeder calves and the management of the time of disposal markedly affect the incidence of respiratory infection. Further, the interstate movement of these animals, although presumably under the influence of Federal regulations and therefore recorded, are almost impossible to trace. The wide range in the movement of these cattle and the diseases that could be and are disseminated from one area to another would call for stricter record keeping on the part of veterinarians, public health officials, and owners.
A. Steps that can be taken at the regulatory level
1. Animals that are subject to interstate movement be marked as to the state of origin
2. Stricter enforcement of Federal regulations regarding the movement of such animals
3. A requirement that recognized professional dealers have legible and permanent records of the animals sold and/or purchased by them for resale. That these records be made available to any prospective purchaser.

B. Steps that can be taken at the rancher-seller level to alleviate conditions where a correlation exists between a management practice and the incidence of respiratory disease.
1. An educational program should be instituted to inform the rancher-seller of detrimental practices.
   a) Use of vaccines at least 30 days prior to sale
   b) Weaning at least 30 days prior to sale
   c) Castration at least 30 days prior to sale
   d) Treatment for grubs at point of origin
2. An educational program should be undertaken to inform the feeder-seller of the pitfalls in obtaining animals
   a) Short transit time
   b) Yard-weary cattle
   c) The increase in respiratory involvement that correlated the surgical, vaccination, and management procedures that occur at time of shipment.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE


The Committee studied several problems that pertain to the eradication and control of infectious diseases of cattle. The Committee wishes to commend the federal and state veterinary regulatory personnel and practicing veterinarians for their continued effort in the eradication of diseases of cattle, specifically brucellosis and tuberculosis. Continued all out effort will be necessary to arrive at complete eradication.

Further, the Committee agreed that the major problems of concern for the members of the United States Livestock Sanitary Association to consider are:

1. Vibriosis in cattle
2. Virus diseases in cattle
   a. Bovine Viral Diarrhea (Mucosal Disease) Complex and other viral agents
   b. Economics of abortion and sterility in cattle due to viral agents
3. Johne's disease control
4. Updating regulations governing procurement and shipment of semen in intrastate and interstate
5. Malignant Lymphoma control
6. Salmonellosis
7. Vesicular diseases

1. Vibriosis in Cattle

Vibriosis is one of the most important diseases of the cattle industry. It is estimated that 50 percent of all beef herds are infected. The Vibrio vaccine now available is a step forward, but there is a great need for improvement in diagnostic tests. Veterinarians in many states do not have services available for the diagnosis of Vibriosis in cattle.

Current emphasis should be on the improvement of the vaccine and diagnostic procedures plus the dissemination of hygienic breeding program recommendations.

2. Virus Diseases of Cattle

Researchers are now untangling the complexity of the Bovine Viral Diarrhea (Mucosal Disease) Complex of cattle. The incidence of this disease is widespread and now is involved in beef and dairy herds as well as in the feedlot.
The existence of other closely related virus diseases besides infectious Bovine Rhinotracheitis and Bovine Viral Diarrhea (Mucosal Disease) Complex need further investigation as to their importance. Further, the importance and extent of these infections must be ascertained in the beef and dairy breeding herds.

Information is also needed in their role as an infectious agent in artificial insemination processes.

3. Johne's Disease is still a problem in breeding herds in the United States. More stringent regulations need to be enforced on movement of cattle infected with the disease. In most states little restriction is placed upon infected individual animal or herd movement.

4. Due to the widespread use of artificial insemination and increased exportation of semen, plus advances in technology, recommended uniform regulations governing all aspects of artificial insemination adopted by the United States Livestock Sanitary Association need constant study and updating when approved changes are necessary. Specifically, the points in question are: (1) improved diagnostic tests for the existence of vibrio in the bulls in the stud. The fluorescent antibody technique needs to be evaluated as a recognized test; (2) the series of tests recommended now to determine the presence of trichomoniasis needs to be evaluated. A more simplified single test is more realistic in that more studs would then comply with the code; (3) the recognition and testing for the presence of viral agents in semen needs to be researched; (4) testing of bulls in studs for different sero-types of leptospira needs to be studied and recommendations given. It was further recommended that all states adopt recommended regulations as approved by the United States Livestock Sanitary Association.

5. Malignant Lymphoma

Again, the Committee feels that regulatory officials should give consideration to this in that this disease is of importance to the cattle industry.

6. Salmonellosis

This disease is of increasing importance to the cattle industry. A special committee should be appointed to cover all phases of the disease.

7. Vesicular Diseases

The fact that Vesicular Stomatitis is difficult to differentiate from other diseases, the Committee feels that regulatory officials should continue to help all field regulatory personnel on the alert for the presence of this disease. Educational efforts should be made to acquaint the livestock industry with this disease. Further it is recommended that the special committee on Vesicular Diseases should be re-established.
Since the 1964 report of the Vesicular Diseases Committee, 370 investigations of suspected vesicular diseases cases have been conducted. The National Animal Disease Laboratory confirmed 247 cases of Vesicular Stomatitis. The last case confirmed in 1964 occurred in late October in Elbert County, Colorado.

From January 1, 1965, to date, 361 cases of suspected vesicular disease have been investigated and 244 cases confirmed in VS. This year, as in 1964, the Indiana type of the virus predominated with 237 cases confirmed in the states of Colorado (87), Arizona (4), New Mexico (145) and Utah (1). The first cases were located in northern New Mexico in Rio Arriba county on July 26th and this area became the focal point of the 1965 epizootic. It should be noted that this area was entirely free of VS in 1964 even though practitioners and regulatory veterinarians were looking for cases having been alerted to the epizootic in south-central Colorado. Last year 182 cases of Indiana VS were confirmed in Colorado compared to 87 cases in western and southern part of the state in 1965.

Only seven cases of the New Jersey type Vesicular Stomatitis have been confirmed in 1965. These occurred in Dallas County, Arkansas, Perry and Coosa Counties, Alabama, Green County, Mississippi and McCurtain County, Oklahoma and Harris County, Texas. This is the first time in several years that the State of Georgia has been free of VS.

The cases confirmed have been about equally divided between the bovine and equine species. Even though confirmed cases in equine were on record, suspected vesicular diseases in cattle in the same area required investigation to rule out Foot-and-Mouth Disease. Situations have been documented where concurrent infection with VS and FMD in equine and bovine led to complacency and the FMD was allowed to spread before it was recognized. We must profit from this experience and maintain our vigilance even though a vesicular disease outbreak involves horses.

This year's epizootic of VS in New Mexico and Colorado brought out the public health aspects of the disease. Late in July, several cases in humans were suspected by veterinarians investigating the reports of vesicular disease in animals. State and Federal public health officials investigated case histories of 24 patients having some epidemiological association with infected animals. Eight patients had signs and symptoms compatible with a clinical diagnosis of VS. Serological studies of acute and convalescent serum samples are being conducted.

Many cases have been documented among laboratory workers and veterinarians who have worked with the virus. The illness may be described as diphasic lasting about one week. The first phase will last from 48 to 72 hours and is marked by general malaise, high fever, and severe headache. There may be a short period of improved condition followed by the second phase of temperature elevation and headache. The second phase may be accompanied by vesicle formation in the mucosa of the lips, tongue, buccal cavity and pharynx. Absence of vesicle formation may confuse the diagnosis with severe influenza being suspected. Livestock owners should be cautioned to handle suspected cases of vesicular diseases with care.
8. *Infectious Keratitis*

Committee recommends that consideration be given to available information in *Infectious Keratitis* and differentiation of this disease from those with similar symptoms.

9. The Committee approved a resolution from Oregon pertaining to the Neonatal diseases of livestock and recommended it be given to the Committee on Resolutions for consideration.
TRIALS AND TRIBULATIONS OF A STATE-WIDE MASTITIS PROGRAM

Dr. A. R. Smith*

Madison, Wisconsin

To set the stage for my discussion, I would like to briefly review the Wisconsin Mastitis Control Program. Dairying in Wisconsin is big business. The cash receipts from sale of milk exceeded 650 million dollars in 1965. It has been estimated that mastitis costs our average producer about $700 a year. Because of this multi-million dollar loss, the program was conceived. All the laws necessary for the program were in effect before the program was planned, and no additional laws have been passed.

To minimize the problems of a program, it must have a single administrator to make final decisions. Wisconsin placed this responsibility in the hands of the Director of Agriculture, Mr. D. N. McDowell. A great many of our problems have been avoided or simplified by his good judgment. He selected an advisory committee of University of Wisconsin and Department of Agriculture personnel which developed a tentative program and an industry committee, reviewed the proposal. The State Board of Agriculture gave final approval. The program went into effect June 1, 1964.

The Animal Health Division and the Dairy, Food and Trade Division of the Wisconsin Department of Agriculture cooperate in our program, which approaches mastitis control from two aspects. The Animal Health Division treats it as a disease problem; the Dairy, Food & Trade Division from a quality milk viewpoint. The latter is the enforcement agency.

Every four months all dairy herds are brucellosis ring tested and catalase tested. The same sample is used for both tests. Results are reported to the farmer on a single form. If the catalase result is 40 percent or higher, a warning notice is sent with the test results to the producer. When the catalase test result is 60 percent and above, or is consecutively over 40 percent, a direct microscopic leucocyte count (DMC) is made.

If the DMC is less than one million, a dairy inspector makes a sanitation inspection of the premises. If bacteria typical of mastitis were observed, or the DMC was one million or more, a regulatory veterinarian visits the farm and does a California Mastitis Test (CMT) of all lactating animals in the herd.

A record of the CMT is given to the owner. On this report are

*Wisconsin State Department of Agriculture, Madison, Wisconsin.
also printed the statutes and regulations covering the sale of milk from sick or diseased animals. Any animal exhibiting clinical mastitis is classified as diseased. Those graded high on the CMT may also be identified as diseased animals. This latter classification is used when it is necessary to reduce the leucocyte count below one million in the herd composite milk. All diseased animals are identified on the report by eartag number. Milk from them must be withheld from the market until such time as they are free of disease. The statutes are very specific regarding sale of insanitary or adulterated milk. It is a criminal offense, punishable by a fine of $200, or imprisonment for six months, or both.

If the producer has been visited by Department personnel, and results are again high, a conference is held by the Area Supervisors of the two Divisions. A review of the producer's record is made. This conference determines the follow-up. The action will be:

1. A sanitation inspection.
2. A CMT of herd.
3. Combination of above two.
4. Milking time inspection.

The last is an unexpected visit by a dairy inspector and a veterinarian. They do a sanitation inspection, observe the milking procedure, and CMT test the cows. If this inspection indicates that the milk is adulterated, or from diseased animals, it must be withheld from the market for human consumption.

To discuss the problems of state-wide mastitis control, let us start where the program does—the sample. Factors affecting sample quality are age, method of collection, and handling. Any mastitis control program must take into consideration the age of the sample. When dealing with mastitis detection in individual herds, sample age is no problem. We can do CMT's at cow side.

A statewide program raised new questions—in proportion to the number of herds involved. In the case of a small number, for example, Florida, with 525 dairy herds, the test could best be done on the individual farm at the bulk tank, to eliminate the effects of aging. The test of choice in such a program would be an estimation of live leucocytes.

In Wisconsin, with 75,000 herds to test, samples must be brought to central pickup stations that we call intakes. They are then transported to one of three laboratories for test the following day. In this case, the sample is at least 24 hours old. Since many farms bulk tanks are emptied every second day, and some are on a three or four day schedule, at some intakes the sample may be considerably older, from 48 to 96 hours.

Why do we consider the age of the sample important? Leucocytes are considered the best indicator of mastitis. They must be alive and intact for some tests. The life expectancy of a white blood cell in milk is limited.
In a large program the collection of samples becomes more complicated. To insure proper sampling and after care, trained personnel should be used. They should be under the direct supervision of the agency in charge of the program.

For a representative sample from a bulk tank, five minutes of agitation is required before collection. The sample should then be refrigerated at 35 to 40 degrees F. until the test is conducted.

In Wisconsin, because of our volume, we must depend on 2,885 licensed milk haulers to collect the samples. Each trucker usually has 15 to 20 producers to pick up in a single day. The five minutes of agitation of the bulk tank then adds up to one hour and 15 minutes, to one hour and 45 minutes of waiting. Will they do it? We can only hope that each one will follow proper procedure.

The Grade B hauler has, up to this point, been interested only in the butterfat sample, which does not require as strict refrigeration. We have had experiences with samples thrown in the cab of the truck, and left for three to four hours. We hope that with experience and more education, we can eliminate most of the poor samples.

As of June 1st of this year, over 50 percent of Wisconsin's milk producers shipped their milk in cans. This number is being rapidly reduced by conversion to bulk tanks for ease in handling, and better refrigeration.

At the 755 can intakes, samples are taken from weigh vats and held under refrigeration until picked up by Department personnel. Collection, identification, and refrigeration are left to the plant personnel. We stress the importance of care in handling, to the plant manager. I have cited these examples to show the number of people involved in sampling.

It may be of interest to some of you that milk supply and demand affect mastitis control programs. When there was a surplus of milk, dairy plants were selective with their producers. The dairyman was forced to produce wholesome milk. There is no longer a dairy surplus. The competition for milk has caused our mastitis program to be overlooked by many of the plants. Too many fieldmen are merely procurers of patrons, with very little thought of increased milk production by mastitis control.

In some states, there are large milk factories with a thousand or more cows. But in Wisconsin, there is not a single herd of that size. The average dairy herd in Wisconsin has between 25 and 30 head. The vast majority of these herds are Grade B producers. In many small herds, we must make follow-up visits. Fifty percent of these follow-ups are to herds with 15 cows or less. A typical owner is 60 to 80 years of age, either hanging on for social security, or supplementing it. He keeps his cows just for something to do! These herd owners are headaches, because production is not great enough for profit to upgrade equipment, and his milking habits are fixed. Economics and time are the only positive cures!
The screening test to use in a program has received considerable study. We will continue our search until satisfied we have found the best. We believe that the test should be economical, simple to run, fairly rapid, and objective as possible, with good repeatability. We have run studies on the various mastitis screening tests. These are our reasons for rejecting others and selecting the catalase test:

1. Modified white side - Variations in individual judgment in grading.
2. The direct microscopic leucocyte count - Slow, tedious, and does not lend itself to large volume.
3. The CMT - Variations in individual judgment in grading the test.
4. The Wisconsin Mastitis Test - Has eliminated the judgment variation of the CMT, but both tests are an estimation of the number of live cells. Here enters the problem of age of sample. Deoxyribonucleic acid (DNA), which is measured in this test, deteriorates with age. In a program where there is a minimum aging of the sample, I believe this is the test of choice today. Age and poor refrigeration do affect the test, and results may be misleading.
5. The catalase test - The Wisconsin screening test. One good feature is that the catalase enzyme does not deteriorate over a period of at least five days. We know it has faults, but we understand most of them. It best fits our need. We use the "syringe method"* for program work.

I seem to be attributing all of our problems to numbers, and this continues with our veterinarians. We have about 450 practitioners in the state, and their varied follow-up techniques approach that number. Some of these men have outstanding programs, complete in every detail. This varies to the other extent with even some opposition.

Uniform guidelines are needed for the control of this disease. This need is not limited to veterinarians, as all groups involved in mastitis control need direction. In drawing up these guidelines, the following must be considered: Preventive medicine, economics, and the fact that it is a herd management problem.

SUMMARY

The problems increase with the size of the program. In a small statewide program, a fresh sample can easily be collected and tested. Larger programs will have to take into consideration the number and size of herds, sampling system and type of screening test.

Streptococcus agalactiae is a widespread pathogen that can cause infectious mastitis often sub-clinical in nature. The infection may remain undetected without laboratory tests. Reduced milk yield, due to infection with this organism, is of serious economic importance. S. agalactiae is the principle cause of mastitis in untreated herds and milk production losses have been estimated as high as 12 percent. Damage to udders from atrophy and fibrosis not only reduces the milk production capacity of infected cows but also shortens the period during which a cow can be maintained economically within a herd.

While S. agalactiae can be differentiated from other streptococci as well as other microorganisms by biochemical tests, the tests required for positive identification would be too time consuming and expensive for large-scale routine identification of herds or individual cows shedding S. agalactiae.

The lytic phenomenon of certain streptococci in the presence of certain staphylococci described by Christie, Atkins and Munch-Petersen, and subsequently named the CAMP test by Murphy et al., established a method adaptable for a culture screening procedure for presumptively identifying herds shedding S. agalactiae.

Christie et al. observed that when sheep or bovine red cells had been altered by beta hemolysin produced by a staphylococcus, certain streptococci growing within an area influenced by the beta hemolysin produced a true hemolysis. They proposed that this phenomenon be used for the identification of S. agalactiae.

The CAMP test must be considered to be a presumptive test for S. agalactiae since there are reports of CAMP-like reactions produced by other organisms. Murphy et al. reported that 14.9 percent of 261 cultures classed as S. uberis gave a positive reaction. Connecticut workers reported CAMP-positive reactions by both S. uberis and S. dysgalactiae. Certain staphylococci and some bacilli also have been reported to produce a CAMP-like reaction. However, those organisms

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The work was supported in part by a grant (EF-AJ-00370) of the Division of Environmental Engineering and Food Protection of the U.S. Department of Health, Education and Welfare.
that are not streptococci but which produce a CAMP-like reaction should be distinguishable from streptococci on the basis of colony morphology.

In 1952, Hovmand published an interesting extension of the CAMP test called the Sun-CAMP test, which was a more rapid screening procedure intended for the identification of *S. agalactiae* from blended herd milk.

A further step in developing a culture medium for identifying *S. agalactiae* useful to bulk milk screening was the introduction of T.K.T. medium in 1953 by Hauge and Ellingsen. Whereas the CAMP test and Sun-CAMP test relied on the production of beta hemolysin during the incubation period, T.K.T. medium incorporated a purified beta hemolysin with the agar so that all the red cells in the medium were altered. In addition, T.K.T. medium contained thallium sulfate and crystal violet (hence the name, T.K.T.: Thallium, Krystal violet, Toxin) making it a selective medium favoring growth of streptococci. The selectivity of the medium is especially valuable for the culture of bulk tank samples, which are likely to yield profuse bacterial growth on non-selective blood agar.

With increased general concern for the production of milk with low cell content and with reduced profit margins realized by producers, it is expected that a wider interest will develop in the identification of those cows and herds shedding *S. agalactiae*. It would seem essential therefore to evaluate culture methods applicable to screening herd production for the purpose of identifying those herds shedding *S. agalactiae*. This study was undertaken to evaluate the use of T.K.T. medium for screening bulk tank milk samples for the presence of *S. agalactiae*.

**MATERIALS AND METHODS**

Milk samples of approximately four ounces were collected weekly from farm bulk tanks by milk truck drivers as previously described. In addition, the milk production of an experimental dairy herd was sampled from a bulk tank frequently. Quarter samples from this experimental herd were collected at monthly intervals for culture and microscopic examination to identify mastitis pathogens and estimate leukocyte content. All milk samples were cultured without previous incubation.

Two culture media were used: Sheep blood agar (five percent citrated sheep blood and 95 percent blood agar base*) and T.K.T. medium (five percent washed sheep red cells, 95 percent modified Edwards medium** and 0.02 gm purified beta hemolysin). All cultures were incubated at 37°C for 24 hours. Streptococcic growth

*Difco Laboratories, Detroit, Michigan.

**Consolidated Laboratories, Inc., Chicago Heights, Illinois.
on blood agar was subjected to the CAMP test. Streptococcic growth on T.K.T. medium surrounded by a zone of complete hemolysis, and CAMP-positive streptococci were presumed to be *S. agalactiae*.

In order to verify the nature of reaction of colonies on T.K.T. medium, 1,032 colonies surrounded by a zone of complete hemolysis from this medium were subjected to the CAMP test. Tests were then conducted to determine the relative efficiencies of a selective and a non-selective culture method for the identification of bulk milk samples containing *S. agalactiae*. In these tests, 1,717 bulk milk samples from 490 herds were cultured. These bulk samples represented herds contributing milk to two milk plants in southern Wisconsin. Approximately 0.01 ml from each sample was cultured on both T.K.T. medium and sheep blood agar. Suspected streptococcic colonies on sheep blood agar were transferred to CAMP plates. Two bulk samples were cultured on each blood agar plate, while four samples were cultured on each T.K.T. plate.

The frequency of isolation of *S. agalactiae* on T.K.T. medium from a bulk tank containing milk from an experimental herd of approximately 40 cows was determined. Within this experimental herd, all lactating quarters had been sampled for culture and microscopic examination at approximately monthly intervals during the last eight years. On these culture examinations, using a non-selective blood agar, no isolations of *S. agalactiae* had been made for a period of four years immediately prior to the present investigation. During this study, infections with *S. agalactiae* were established on a controlled scale as verified by continued monthly examination of quarter samples. Initially, one quarter each of two cows was infused with a culture of *S. agalactiae* isolated from a commercial herd within the previous month. Infection was assumed to have been established when quarter samples collected six to eight milkings after infusion yielded CAMP-positive streptococci. At intervals of approximately one to three weeks, additional quarters were similarly infused, two at a time.

Ten replicate samples, one ounce each, were collected from the bulk tank following five minutes of agitation at irregular sampling periods that ranged from daily to several days apart. All bulk milk samples in this study were cultured on T.K.T. medium immediately following collection.

RESULTS

Of the isolates positive on T.K.T. medium from 1,032 bulk samples, there were 990 (95.9 percent) that gave a characteristic positive reaction on the CAMP test.

Culture of bulk milk on T.K.T. medium identified nearly twice as many herds shedding *S. agalactiae* as culture on a non-selective medium. One thousand seven hundred seventeen bulk milk samples
from 490 herds were cultured on T.K.T. medium, as well as non-selective blood agar and all suspected streptococccic colonies on blood agar were subjected to the CAMP test. The selective medium identified 434 herds as shedding S. *agalactiae*, while the non-selective culture method identified 232 (Table I).

**TABLE I**

Comparison of Two Culture Methods for Identifying Herds Shedding *Streptococcus agalactiae*

<table>
<thead>
<tr>
<th>Milk shed</th>
<th>Herds contributing samples (no.)</th>
<th>Samples contributed (no.)</th>
<th>Herds with positive results (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CAMP 123</td>
</tr>
<tr>
<td>D</td>
<td>227</td>
<td>949</td>
<td>216</td>
</tr>
<tr>
<td>W</td>
<td>263</td>
<td>768</td>
<td>109</td>
</tr>
<tr>
<td>Totals</td>
<td>490</td>
<td>1,717</td>
<td>232 (47%) 434 (89%)</td>
</tr>
</tbody>
</table>

In an experimental herd of approximately 40 cows, it was necessary to have more than five percent of those quarters contributing to the bulk tank, shedding *S. agalactiae* in order to isolate the organism on T.K.T. medium from the culture of a single bulk tank sample. When 5.3 percent of the quarters were shedding *S. agalactiae*, isolations of the organisms were made from 84 percent of 160 bulk tank samples (Table II).

In relation to the isolation of *S. agalactiae* on T.K.T. medium from bulk tank milk from an experimental herd, it is of interest to examine the distributions of reactions on T.K.T. medium of those bulk samples contributed by the 490 herds described earlier. Every sample contributed by 358 of the 490 herds was positive on T.K.T. medium. There were some positive and some negative samples from 108 herds, while 24 herds contributed only samples that were negative on T.K.T. medium (Table III). All but 48 of the herds contributed two or more samples. In a further breakdown of the distribution of samples from those herds that contributed both positive and negative samples, it was found that more samples were positive than negative.

**TABLE II**

Isolations of T.K.T.-positive Organisms from a Bulk Tank Containing Milk from an Experimental Herd with a Controlled Number of Quarters Infected with *Streptococcus agalactiae*

<table>
<thead>
<tr>
<th>Cows (no.)</th>
<th>Quarters (no.)</th>
<th>Quarters infected no. %</th>
<th>Bulk tank samples (no.)</th>
<th>T.K.T. positive samples no. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>154</td>
<td>2 1.3</td>
<td>50</td>
<td>3 6.0</td>
</tr>
<tr>
<td>41</td>
<td>161</td>
<td>4 2.5</td>
<td>50</td>
<td>18 26.0</td>
</tr>
<tr>
<td>39</td>
<td>153</td>
<td>6 3.9</td>
<td>100</td>
<td>24 24.0</td>
</tr>
<tr>
<td>38</td>
<td>150</td>
<td>8 5.3</td>
<td>160</td>
<td>135 84.4</td>
</tr>
</tbody>
</table>
TABLE III

Distribution by Farms of Samples Contributed by 490 Herds Based on Number of Samples Contributed with the Indicated Results from Culture on T.K.T. Medium

<table>
<thead>
<tr>
<th>Samples cultured on T.K.T. medium</th>
<th>Total Samples Contributed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>All positive</td>
<td>39</td>
</tr>
<tr>
<td>Some positive, some negative</td>
<td>0</td>
</tr>
<tr>
<td>All negative</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
</tr>
</tbody>
</table>

DISCUSSION

The efficiency of a selective medium for identifying *S. agalactiae* in bulk milk from a geographically limited sample area was demonstrated in this investigation. The surprisingly high percent of herds identified as shedding *S. agalactiae* raises interesting speculative questions about other areas of high concentration of dairy cattle. It would be of interest to extend the sampling area to include bulk milk from herds under a periodic quarter sampling program for the identification of infected cows and herds.

The four percent of the colonies that were positive on T.K.T. medium that were not CAMP-positive in this study must remain unexplained, as they were not subjected to further identification. Presumably, some of this lack of agreement could be attributed to technical error in the identification and isolation of colonies.

The advantage of blood agar for the culture of bulk milk samples for the isolation of many mastitis pathogens on a single culture medium is apparent. However in this investigation, nearly half of the herds shedding *S. agalactiae* would have remained unidentified without the use of a selective medium favoring streptococci.

Herds negative to initial culture may be retested with minimal expense. Each T.K.T. plate used in this investigation cost approximately four cents, excluding labor. By culturing four samples on one plate, bulk milk samples may be screened for *S. agalactiae* at a material cost of approximately one cent.

ACKNOWLEDGMENTS

The author wishes to express appreciation to Miss Karen Mandt for technical assistance, and to Deerfield Creamery Company and Wisconsin Dairies Cooperative for supplying bulk milk samples.
REFERENCES


REPORT OF THE COMMITTEE ON MASTITIS

K. J. Peterson, Corvallis, Oregon, Chairman; R. K. Anderson, St. Paul, Minnesota; T. M. Birch, Bloomer, Wisconsin; H. S. Bryan, Kalamazoo, Michigan; R. I. Hostetler, Pullman, Washington; E. J. Kersting, Storrs, Connecticut; D. S. Postle, Madison, Wisconsin; R. J. Schroeder, South Gate, California; J. V. Smith, Hartford, Connecticut

The Mastitis Committee has met twice during the year. The first meeting was held in Louisville, Kentucky July 12, 1966 during the National AVMA Convention and the second October 11, during this 1966 annual United States Livestock Sanitary Association meeting in Buffalo.

Since 1960 numerous municipalities and milk processing plants, and recently several states, have initiated milk leukocyte testing programs. Samples are periodically collected from each producers' milk supply, usually from the bulk tank, and subjected to either the direct microscopic leukocyte count, the California Mastitis Test, Modified Whiteside Test, Wisconsin Mastitis Test, or the catalase test. Programs vary to some extent, but milk containing leukocytes in excess of one million per ml. is generally considered undesirable. The producer is notified, informed of the high count and instructed to immediately take necessary steps to reduce it. Since mastitis is usually the cause of the excessive leukocyte count, it is generally suggested that the milking equipment field man carefully check the milking equipment and that a veterinarian be consulted.

Although such milk testing programs are often referred to as mastitis control programs, they are in reality, milk quality control programs and should be so designated. A dairyman may immediately reduce the bulk tank leukocyte count by removing from the milking string cows shedding high numbers of leukocytes. This satisfies those conducting the test but does not solve the herd mastitis problem. Conscientious dairymen may be experiencing serious losses from chronic mastitis but by following the recommended practice of removing infected cows from the milking string, their herds may never be identified as problem herds. Serious herd losses from acute mastitis may also go undetected since mammary secretions from acutely ill cows are rarely allowed in the milk supply.

The committee realized the benefits derived from milk quality control programs and recommends the continuation and expansion of these programs. However, bovine mastitis is an extremely complex infectious disease and for best results, prevention and control programs must be under the direct supervision of men within the veterinary profession. Each infected herd is an individual problem: 1) predisposing causes must be identified and eliminated; 2) chronic incurable infected
cows must be located and culled; 3) the causative organism or organisms must be isolated and identified, (drug sensitivity testing may be necessary); 4) methods of spread must be determined and 5) carefully planned sanitation and treatment programs must be initiated. These procedures require a thorough knowledge of disease control.

The committee urges that all veterinarians concerned with diseases of dairy cattle, develop greater interest in mastitis prevention and control. Increased veterinary attendance and participation at local, county and state mastitis meetings is sorely needed.

The committee recommends that (1) the United States Livestock Sanitary Association continue it's membership in the National Mastitis Council and that it participates more actively in the functions of this organization. Improved liaison between these two organizations would be beneficial. The committee urges the chairman of the United States Livestock Sanitary Association mastitis committee to attend the annual meeting of the National Mastitis Council. If he is unable to attend, another committee member should substitute for him. (2) It is recommended that standard technical nomenclature be used to describe screening tests, culture methods, and diagnostic procedures. We recommend that a subcommittee be appointed to develop and recommend standard nomenclature. (3) It is recommended that committee member Dr. D. S. Postle attend the joint meeting of the National Conference of Interstate Milk Shippers and the National Mastitis Council, November 17, 1966, in Chicago, Illinois and report on the activities of this meeting to the mastitis committee. (4) Recent increased emphasis upon mastitis control makes it desirable and necessary to maintain committee continuity. It is therefore recommended that the present chairman and members remain on the mastitis committee for one more year. Committee members should then be appointed on a staggered term basis with each member serving three consecutive years.
STUDIES ON THE BLOOD CHEMISTRY OF CATTLE IN A HERD INFECTED WITH JOHNE’S DISEASE

Ames, Iowa

REVIEW OF LITERATURE

Reports of blood chemistry studies in cattle in herds that are infected with Johne’s disease are rather limited. Fouquet and Delauney in France reported that herds heavily infected with Johne's disease were located on farms with soils deficient in phosphorus. They also reported that the addition of mineral supplements rich in phosphorus to the ration greatly reduced losses from the disease and that beneficial therapeutic results were obtained when phosphorus containing compounds were injected intravenously in cattle showing clinical signs of disease. Stewart et al. reported that 19 sheep and nine cows with clinical signs of Johne’s disease had low calcium and magnesium and normal phosphorus levels except two cows that appeared to have high phosphorus levels.

Phosphatases are enzymes that hydrolyze orthophosphomono esters with the liberation of inorganic phosphate and since alkaline phosphatase is closely associated with phosphorus metabolism it was decided to make determinations of this enzyme as part of our study. Wise et al. found that when normal calves were placed on a phosphorus deficient diet, alkaline phosphatase activity increased as the serum level of phosphorus decreased but after a two-week period, the activity of the enzyme approached normal levels. Patterson et al. found that the levels of activity of this enzyme in cattle with Johne’s disease was in approximately the same range as that found in normal cattle. Merkal, using histochemical procedures found more alkaline phosphatase in tissues of sheep that were infected with Mycobacterium paratuberculosis than was present in similar tissues in noninfected sheep. Elevated levels of alkaline phosphatase have been found in serums from humans with certain bone diseases and with liver abscesses.

This paper reports the results of a study of phosphorus, calcium, sodium, potassium and magnesium levels and phosphatase activity of serums from cattle in a herd infected with Johne’s disease and compares these levels with published reports of normal levels.

From the National Animal Disease Laboratory, ADP Research Division, Agricultural Research Service, U. S. Department of Agriculture, Ames, Iowa.

The authors acknowledge the assistance of Ed Carney, Iowa State University in the statistical analysis and Dr. E. T. Littledike, NADL for making the magnesium determinations.
Two-hundred fifty-eight adult Guernsey cattle in a herd in which Johne's disease was an economic problem were selected for a two-year study. These cattle were part of a dairy herd and it was planned for each cow to calve at approximately 12-month intervals. All cattle were born on the farm and ranged in age from four to 11 years. To assist in developing the data, the cattle were divided into three groups as follows: (1) Noninfected cattle, (2) infected cattle showing no clinical signs of disease and (3) infected cattle showing clinical signs typical of Johne's disease. The herd obtained most of their roughage from pasture consisting of carpetgrass (*Axonopus affinis*) bahiagrass (*Paspalum notatum*) and white clover (*Trifolium repens*). A commercial concentrate was used to supplement the roughage. In addition to protein, fiber, fat and carbohydrate the concentrate contained two percent added minerals. These consisted of sodium chloride, defluorinated phosphate, calcium carbonate, zinc oxide, iron oxide, manganese sulphate, copper sulphate, cobalt carbonate, potassium iodide and sodium bicarbonate.

A blood sample was obtained from each cow at six-month intervals. Serum was obtained by centrifugation and stored at -56°C until the analytical determinations were made. Phosphorus was determined by the method described by Fiske and Subbarow, calcium by a method described by Roe and Kahn, and alkaline phosphatase activity by the method of Bodansky. Sodium and potassium were determined by flame photometry.* Magnesium was determined with an atomic absorption spectrophotometer.**

Normal attrition resulted in the slaughter of approximately 60 of these cattle each year and since serum samples were obtained at six-month intervals, postmortem examinations were made from one day to six months following the last serum sample. A portion of the intestinal tract including the ileocecal valve was obtained from each slaughtered cow and examined for the presence of *Mycobacterium paratuberculosis*. Smears were prepared following trypsin digestion of the tissues and stained by the Ziehl-Neelsen procedure. In addition, cultures were prepared from the trypsin digest. If acid-fast bacilli morphologically or culturally indistinguishable from *M. paratuberculosis* could not be observed in smears of cultures, the animal from which the specimen was obtained was classified as "noninfected." If such bacilli could be demonstrated but the animal from which it was obtained showed no clinical signs of disease, the animal was classified as "infected." If the bacilli could be demonstrated and, in addition, the animal was showing clinical signs typical of Johne's disease it was classified as "clinical." These cattle were slaughtered within 30 days of the date that clinical signs were first observed. The data were subjected to statistical analysis. Significance tests of the difference between the means were made at the five percent level using the Welch approximate t-test.

*Model B Spectrophotometer, Backman Instruments, Inc., Fullerton, California.
**Perkin-Elmer Model 290.
**TABLE I**

Determination of Serum Levels of Phosphorus, Phosphatase, Calcium, Potassium, Magnesium and Sodium in Cattle from a Herd Infected with Johne's Disease

<table>
<thead>
<tr>
<th>Determinations made 6 months or more before slaughter</th>
<th>Determinations made within 6 months of slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cattle</td>
<td>Ca mg/100 ml</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Negative on postmortem examination</td>
<td>54</td>
</tr>
<tr>
<td>Positive on postmortem examination but showing no outward signs of disease</td>
<td>46</td>
</tr>
<tr>
<td>Clinical signs of disease and positive on postmortem examination</td>
<td>20</td>
</tr>
<tr>
<td>Values for normal cattle</td>
<td></td>
</tr>
</tbody>
</table>

*Handbook of Biological Data, National Academy of Sciences and National Research Council.
**Determined from normal adult cattle at the National Animal Disease Laboratory.
***Clinical Biochemistry of Domestic Animals, Academic Press, N. Y.
RESULTS

The results are shown in Table I. It will be observed that values obtained from serums of noninfected cattle and infected cattle not showing clinical signs of disease were very similar and also similar to published reports of normal values.

The values obtained in the group showing clinical signs of disease were similar to those of the other two groups except for the phosphorus level and phosphatase activity. The phosphorus levels in serums from those showing clinical signs of disease had an average increase of about 35 percent over previous determinations and were also about 35 percent higher than the values in the other two groups. This was found to be statistically significant at the five percent level. Phosphatase activity was also higher in most of the cattle in the group showing clinical signs of disease than it was in the other two groups. This was also statistically significant at the five percent level.

DISCUSSION

Cattle in this herd were on a diet containing adequate quantities of phosphorus, calcium, sodium, potassium and magnesium. Therefore, no therapeutic effect could be expected from feeding additional mineral supplements. It is thought that adverse conditions may cause normal appearing cattle infected with Johne's disease to develop clinical signs of the disease. However, a controlled experiment on nutritional levels and stress is needed to provide reliable information in this regard. It is possible that a mineral deficient diet would cause an increase in the number of cattle showing clinical signs but the results of our observations indicate that reportedly adequate levels of these elements in the diet does not eliminate the disease.

It is not known why there was an increase in alkaline phosphatase and phosphorus in the serums of cattle showing clinical signs of disease. Apparently advanced infection in the tissues causes an increase of alkaline phosphatase which in turn increases the inorganic phosphorus level in the serum.

SUMMARY

Blood chemistry studies were made in a herd of cattle infected with Johne's disease. All cattle were examined physically for clinical signs of the disease and bacteriologically at postmortem for evidence of infection.

Serum levels of inorganic phosphorus, calcium, sodium, potassium and magnesium and alkaline phosphatase activity were determined. It was found that the values obtained from noninfected cattle and infected cattle not showing clinical signs of disease were similar to published values for normal cattle, but that infected cattle showing clinical signs of disease had higher levels of phosphorus and phosphatase activity than normal cattle.
REFERENCES

THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

A. F. Ranney, D.V.M., M.S.*

In a disease eradication program the principle of comparing past accomplishments with the present situation is basic to future planning.

The stubbornness of tuberculosis infection was less apparent when more spectacular progress was being made in reducing the incidence of the disease than today when scattered foci of infection are difficult to seek out and eliminate. The attack on tuberculosis has always encountered problems many of which have been overcome with experience and new knowledge. Others are being solved as we continue to learn while we eradicate.

Many people and groups have continued to contribute much to the State-Federal Cooperative Tuberculosis Eradication Program during the past year. The part that meat inspectors play in this program and their interest in tuberculosis eradication has been discussed by Leighty of the United States Department of Agriculture, Consumer and Marketing Service.¹ Meat inspectors (State and Federal) reported finding in cattle approximately 800 cases of tuberculous lesions or lesions resembling tuberculosis. Much of the success in locating source herds was based on descriptive identification of subject animals furnished by meat inspectors. Many of the packing plant officials recognized the importance of maintaining, through slaughter, descriptive animal identification. This made it possible for the inspector to provide the necessary information in his report to the State-Federal cooperating officials.

Personnel at the National Animal Disease Laboratory provided important information on the results of pathological and bacteriological examinations on specimens submitted by meat inspectors. These laboratory results are an essential part of our epidemiologic studies.

The National Tuberculosis Association and affiliated local organizations gave a generous amount of their time by participation in work conferences in various geographical areas. The exchange of information on experiences in tuberculosis eradication in humans and domestic animals was beneficial to the participants.

Veterinarians who are engaged in disease eradication among animals at zoological gardens also participated in the work conferences on eradication procedures. Thus, in setting a course for final eradication of the disease, action is being taken to simultaneously eradicate it from other related species to eliminate reservoirs of infection.

It is impossible to include here all those who have contributed their time and talents to this program and to whom we are indebted.

*Chief Staff Veterinarian, Tuberculosis Eradication, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
The steady reduction in the number of reactor herds reported with infection indicative of *Mycobacterium bovis* during the past five years is illustrated in Figure 1. This certainly suggests progress although we must watch carefully to insure that the procedures are adequate to locate infection more rapidly than it is spread. As the incidence of bovine tuberculosis drops, area retesting becomes relatively more costly as a long-term surveillance procedure. However, we must consider the possibility that the decline in the number of infected herds reported and shown on the chart may not coincide with the actual field situation.

Of the 111 herds in which *M. bovis* was reported during fiscal year 1966, 56 percent were first detected as a result of traceback testing, while 44 percent were found as a result of routine testing procedures. The comparison of infected herds found by the two general procedures for each of the past five years is shown in Figure 2. Fiscal year 1966 was the first year that a higher percentage of infected herds were located as a result of traceback testing as compared to routine testing.

A review of infected herds found as a result of tracing, Figure 2, shows that the number has remained fairly constant while there has been a drastic reduction (173 to 94) in the number of herds found as a result of routine testing procedures. We might speculate as to whether this
reduction is primarily due to the 38 percent decrease in volume of area testing between the years 1962-66 (5.1 million to 3.1 million).

The number and percentage of infected herds reported for the past year is further broken down in Figure 3 to show specific reasons for initial tests under the two main categories, routine testing and tracing. The greater portion of infected herds found as a result of tracing as compared to routine testing can be attributed in some degree to continued emphasis on epidemiological procedures.

During fiscal year 1966 a total of 6,702,017 cattle were recorded as tested for routine purposes. This figure divided by the number of herds (49) initially located as a result of routine testing gives an average of about 137,000 tests to locate an M. bovis herd. On the other hand, the 60,375 cattle tested last year following traceback divided by the number of herds (62) initially found by traceback testing gives an average of slightly less than 1,000 cattle tested to locate an infected herd.

A comparison between routine testing and testing following traceback procedures as a means of locating infected herds is shown in Figure 4. It is of interest to note that the same number of herds with early infection (32) were found under each category while the relative difference between finding early and advanced infection is considerably greater in the case of
routine testing as compared to tests following traceback procedures. These data suggest that routine testing even though relatively costly should be maintained in selected areas as an essential part of the program unless or until other means of detecting early infection are more productive.

The breakdown of the 111 herds into types of cattle located as a result of routine testing and traceback testing is portrayed in Figure 5. The percentage figures for dairy herds of 53 percent and 47 percent for routine and traceback testing respectively is quite a contrast to the 25 percent and 75 percent figures for beef-type cattle. The greater difference in percentage figures for tests made in beef-type animals is undoubtedly due in part to the fact that area testing is being continued to a greater degree in areas where dairy cattle predominate as compared to areas engaged primarily in beef cattle production.

The following statement taken from "A Report to the Surgeon General of the Public Health Service by a Task Force on Tuberculosis Control," is worthy of consideration in eradicating tuberculosis from animals as well as people: "In most of the country, identification and repeated examinations of persons at risk should replace mass screening techniques that are no longer productive. . . . Blame for the slowing
down of progress against tuberculosis undoubtedly lies, in part, with over-optimism about what would be needed to finish the job. Realistic assessment of the situation and measures to increase and improve control efforts are in order."

All too frequently adequate positive steps are neglected when history and the evidence at hand tells us to apply drastic eradication measures. Failure to carry out these measures that may at the time seem severe is delaying the eradication of bovine tuberculosis. This results in severe losses to some herd owners and increased expenditure of tax dollars. In eradicating tuberculosis from livestock we are still depending on mass screening techniques. The degree to which we can profitably change from mass testing in selected areas to the identification of cattle disclosing tuberculous lesions by meat inspectors at time of slaughter as the sole screening technique, coupled with thorough epidemiological investigation of the infected herd, must be carefully weighed.

Due to the insidious nature of tuberculosis, there has been a problem of continuous infection in some herds and the reappearance of the disease in others. The change in Federal regulations in 1964 to provide for indemnity payments for exposed non-reacting animals in selected herds has stimulated the depopulation of herds with heavy or repeated infection. A
comparison of herds with infection reported during 1965 and 1966, which also had infection in a prior year, is shown in Figure 6. The decrease from 44 percent to 25 percent may be attributed in part to depopulation of herds with long standing infection.

The 28 herds with *M. bovis* infection in 1966 that also had infection in a prior year have been summarized in Figure 7 to show the year or years in which infection was previously reported. A summary of the current status of herds involved is also shown. It may be observed that in one case a herd had infection reported in nine different years over a period of 16 years before the entire herd was sent to slaughter. In another herd, infection was found in each of five years during the past seven. This herd is shown among those presently under quarantine.

Of the 28 herds, eight have been quarantined once, 11 quarantined twice, seven quarantined three times and two were quarantined for the fourth time. It is impossible to estimate the amount of infection that may have been spread between quarantine periods.

While in some of these herds requarantine may be due to reinfection in most herds, tuberculosis remained like a smouldering fire.

It has been pointed out in prior reports to this Association that the elimination for slaughter of herds with repeated infection and those
Tuberculosis Eradication

Herds Reported with infection (Indicative of M. bovis)

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>1965</th>
<th>1966</th>
</tr>
</thead>
<tbody>
<tr>
<td>141</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>44.68%</td>
<td>63</td>
<td>28</td>
</tr>
<tr>
<td>25.23%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No Record Prior Infection vs Infection Reported in a Prior F. Y.

Figure 6

Tuberculosis Eradication

RECURRENT INFECTION REPORTED IN 28 HERDS

<table>
<thead>
<tr>
<th>No. Infected Herds</th>
<th>FISCAL YEAR OF RECURRENT INFECTION</th>
<th>CURRENT HERD STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1956 '55 '54 '53 '52 '51</td>
<td>IN QUARANTINE</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
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Figure 7
found to be heavily infected is a highly effective eradication procedure.

A review of records clearly show that it is more economical in the long run to depopulate many of these herds than to attempt to eliminate the disease by repeated testing. This is especially true since we are working with a very low level of infection and approaching the final goal.

The 111 reactor herds with infection indicative of *M. bovis*, previously referred to, were located in 22 States and Puerto Rico. The numbers reported in each State for the past year are outlined in Figure 8. The numbers shown on the outside of the map of the country pertain to the total number of herds reported for each of the four regions as denoted by the heavy lines. In looking at a map of this kind, questions are frequently raised and fingers pointed at the States with the larger numbers. This deduction is often most unjust. In a majority of cases, the States with a high number of infected herds are among those where there is a vigorous cooperative State-Federal eradication program. This makes the number of infected herds stand out because they are detected. The officials are well aware of the fact that strenuous efforts now will pay dividends in the future.

To illustrate one outstanding case among many has been selected to depict effective traceback and follow-up on a herd that was apparently
heavily infected at the time of dispersal. Several important points may be gleaned from this case (Figure 9): (1) The State-Federal officials in Ohio took prompt and positive action in this epidemiological study. (2) Two animals that originated from a common source were found about the same time by two different procedures. The animal (herd A) in which lesions were found on regular kill meat inspection, January 18, 1966; and the animal (herd B) that reacted on an area test two days later, January 20, 1966, were both traced to a herd of 91 animals that was dispersed four months previously, September 16, 1965. (3) The 91 animals from the dispersed herd were sold into 24 herds. (4) Eleven herds had tuberculous animals that originated from the subject herd. (The two, A & B, that prompted the case study and the nine herds in which infection was found on follow-up.) (5) There were nine herds to which 11 animals had been added from the subject herd where no infection has been reported to date. (6) Ten animals sold into five herds were reported sold for slaughter before tracings and tests were made. One of these was to one of the nine herds where another addition was tested but no infection found. (7) Eight animals were sold interstate to a dealer where their identity was lost. With the amount of infection found in the successful tracings we can only speculate as to: (a) the number of tuberculous animals among the eight, (b) the

OUTSTANDING TB TRACEBACK

HERD SALE
91 ANIMALS
SEPT. 16, '65

SLAUGHTER PLANT
JAN. 18, '66

TB DISCOVERED..TRACED BACK TO... 

AREA TEST
JAN. 20, '66

HERD A
13 ANIMALS

HERD B
16 ANIMALS

8 ANIMALS TO INTERSTATE DEALER
IDENTITY LOST

10 ANIMALS TO SLAUGHTER
OUT OF 5 HERDS

11 ANIMALS TESTED
NEGATIVE OUT OF 9 HERDS

33 ANIMALS INFECTED 9 HERDS

AGRICULTURAL RESEARCH SERVICE

Figure 9
number of herds to which they may have gone to spread infection, (c) the number of months or years before additional infected herds may be discovered as a result of the sales of these eight exposed animals, (d) the number of years that this may delay the eradication of tuberculosis from the States involved, (e) the degree of hazard to human health, (f) the financial loss to the livestock owners whose herds may become infected; and (g) the tax dollars that must be added to the program as a result of this potential spread of infection.

Some question may arise concerning the word "progress" which is included in the title of the graph (Figure 10), as there has been an increase in the number of Red Flag Herds reported. This is the first year that there has been an upswing in the number of herds in the "Red Flag" category since the term was applied to herds with repeated lesion reactors. This suggests progress when we realize that some of the infections were located after a more active local program was initiated. This chart emphasizes the need for continued vigilance in our attack.

The States in which these 21 herds are located are shown on the map (Figure 11.) The relative period of time that these herds have been infected is illustrated.

At the present time, there are 606 counties or districts in 23 States, Puerto Rico and the Virgin Islands listed as accredited tuberculosis free
Figure 11

(bovine type TB in cattle). This designation has had a stimulating effect in tuberculosis eradication activities. The need for continued vigorous assault on our age-old enemy is depicted on the map (Figure 12), where areas are coded to show where cattle with infection positive or strongly suspicious of M. bovis have been discovered since free status was attained. Involved are 31 cases in 28 counties.

During the past year, outbreaks of bovine tuberculosis involving humans and animals other than cattle are worthy of note. The following cases are among those that have come to our attention:

1. Tuberculosis (M. bovis) was found in a herd of 15 elk at a game preserve in Pennsylvania. The entire herd was slaughtered.

2. M. bovis infection in wild swine was reported last year on a large California ranch where tuberculosis in cattle has been a problem. The State-Federal cooperating officials have arranged for these swine to be hunted and trapped by experienced personnel from the Bureau of Sports, Fisheries and Wildlife, Department of Interior.

3. After locating extensive tuberculosis in a Pennsylvania dairy herd following a meat inspection lesion report, generalized lesions of tuberculosis were found in an 18-year-old pet goat. This goat had been in close contact with the cattle.
4. A fatal case of infection due to *Mycobacterium bovis* involving a pharmacist from Connecticut was described in May 1963.  

5. A dairyman in New York State, who lost his entire herd of 47 cattle in 1947 due to tuberculosis, was found to have an active case of bovine type tuberculosis. Following disclosure of the disease in this man, his herd of 100 cattle was tested. Fourteen reactors were revealed.

For the purpose of solving the problems associated with "non-specific sensitivity, several special studies have been conducted or initiated which include:

1. **Use of PPD rather than heat concentrated synthetic medium tuberculin.** There was no statistically significant difference found in the specificity of Weybridge PPD as compared to the United States Department of Agriculture Standard Tuberculin.

2. **Use of dilute tuberculin.** Dilute tuberculins (1/10 and 1/100) were effective in reducing the number of non-specific responses. However, the number of infected animals that were missed was increased to the point where some infected herds would have been skipped entirely.

3. **The role of trace-mineral imbalance on the sensitization of cattle.** Work is incomplete on this project but preliminary results suggest the possibility of copper and phosphorus imbalances being responsible
for increased susceptibility to anonymous mycobacterial infections which result in tuberculin sensitivity. This sensitivity appears relatively constant on a herd basis but rotates among the members of the herd.

4. Role of skin lesions in tuberculin sensitivity. Work has just been initiated on two aspects of this problem. First, is an ecologic study to determine whether or not the occurrence or absence of skin lesions is related to soil type (i.e., sandy clay, elevated and drained, low with or without drainage, etc.). Insufficient work has been done to evaluate results. Second, is a complete ecologic study which has just begun which will include study of management practices, feeds and water.

5. Use of serological tests. The complement-fixation test was found to be much less specific than the tuberculin test when evaluated in both tuberculin hypersensitive herds and herds completely free of tuberculin responses. The gel-diffusion precipitin test was found to be incompatible with present testing procedures in that tuberculin tests appear to elicit precipitin production in non-tuberculous cattle. A search for a better antigen is presently underway.

6. The comparative cervical test. This consists of comparative intradermal tests in the cervical area using mammalian tuberculin simultaneously with avian tuberculin or tuberculin produced from various anonymous mycobacteria. Tests using all four of Runyon's Groups have been conducted and will be continued. So far a completely satisfactory comparative tuberculin test has not been found. The most satisfactory product to date has been avian tuberculin. Local differences have been noted; however, in some specific locations it has proven to be highly unsatisfactory in that the mammalian response is consistently larger in the absence of an evidence of tuberculosis on postmortem examination or culture. A broad nationwide evaluation of the use of avian tuberculin for this purpose is presently underway.

In addition to the above studies a number of studies on tuberculosis in other species of animals and of Johne's diseases in cattle are being conducted. Other species being studied are:

1. Horses: In order to determine the prevalence of tuberculin sensitivity and the nature of the sensitizing organism.
2. Dogs and cats: To determine the optimal diagnostic procedure. X-ray, tuberculin test and BGG test, as well as sputum examination, are being studied.
3. Swine: In order to determine the best approach to eradication and the nature of the infecting organism. Although M. avium is a significant pathogen of swine, Runyon Group III mycobacteria is emerging as being of almost equal significance. There is a question as to whether some of the Runyon Group III organisms are merely attenuated strains of M. avium.
4. Johne's disease studies are centered on an evaluation of vaccination as a control measure and the effect of Johne's vaccination on the tuberculin test.
This organization should maintain an awareness of avian type tuberculosis as it applies to disease in domestic animals and man. *M. avium* has been reported in many species of animals. For many years a high percentage of the lesions of tuberculosis reported in swine have been considered to be associated with *M. avium*. From 10 to 20 percent of the tuberculous lesions from cattle submitted to the National Animal Disease Laboratory (NADL) and from which acid-fast organisms have been cultured and types are reported as *M. avium*. Of the 165 tuberculous lesions from regular kill cattle from which mycobacteria were isolated at NADL during fiscal year 1966, 84 or 50 percent were classed as *M. avium*.

*M. avium* has been reported as coming from cattle that have reacted to the Johnin test and in which conclusive pathological or bacteriological evidence of Johnne's disease has been lacking. From a public health standpoint, a recent review of avian in man is of special interest. "The seriousness of the disease's clinical course and its prognosis is a weighty reason for eliminating the natural sources of avian tuberculosis, particularly in regions where the human population is no longer exposed to widespread infection by mammalian mycobacteria."

There has been much speculation on the relationship of *M. avium* and Rynyon's Group III organisms. Wayne makes the following statement: "It is proposed that Group III organisms of the Battey type be considered *M. avium* rather than a separate species." If this proposal is more generally accepted, the interrelationship of *M. avium* in livestock and humans is bound to increase in significance.

Our eradication efforts must be strengthened by applying more diligently knowledge already at hand and taking advantage of new scientific information that may be pertinent to the program. "The road to success is always under construction."

REFERENCES

REPORT OF THE COMMITTEE ON TUBERCULOSIS
AND PARATUBERCULOSIS


1. The mid-year meeting of the Committee was held immediately following that of the North Central group in Chicago.

2. It had been proposed to utilize the time at the mid-year meeting for a seminar of Johne's disease to be led by Dr. Aubrey Larson who is in charge of Johne's research at the National Animal Disease Laboratory, Ames, Iowa. However, Dr. Larson stated that he was involved in some important research on Johne's disease which was not yet completed; therefore, he requested that the symposium be postponed until the annual meeting in Buffalo, at which time he was sure his experiment would be completed.

3. A letter from Dr. William Bendix, State Veterinarian of Virginia, was read before the Committee. This letter requested several changes in the uniform method and rules. It was decided that because committee members had not had time to evaluate the letter that this discussion would take place at the fall meeting of the United States Livestock Sanitary Association, with a decision on the recommendations rendered at that time. Upon the evaluation of the recommendations for revision of the uniform methods and rules as brought forth by Dr. Bendix of Virginia, the Committee recommends that the changes be made at the 1967 meeting, because uniform approved laboratory procedures for the diagnosis of tuberculosis have not been completed at this time.

4. The Committee again urges the state and federal livestock sanitary officials to continue to develop the identification and traceback program to its optimum efficiency. Primarily to increase the surveillance for tuberculosis, as well as to relieve "down the road" testing to a greater degree.

5. The subject of individually accredited herds for tuberculosis was brought up and it was suggested that this accredited herd status be discontinued. However, it is the general feeling of the Committee that even though the numbers of accredited herds are small compared to the total national cattle population that it does indicate individual owner responsibility for herd health and serves to stimulate this
responsibility in high class breeding herds. It is further felt that accredited herd owners, as a result of their individual concern for health, have achieved and enjoyed a great deal of integrity as to the quality of their breeding animals. Therefore, the Committee feels that the status of the individual accredited herds be continued as is outlined in the uniform methods and rules.

6. Inasmuch as tremendously valuable progress has been made on tuberculosis research in the last few years the Committee recommends that this research be continued.

7. One of the states has again requested that the Committee give consideration to the reaccreditation of areas on a state-wide basis. The Committee can only refer back to the report of this Committee in 1965. They felt that this could be accomplished within the scope of the present uniform methods and rules. The Committee further recommends that in the future states not apply for tuberculosis free status until the entire state qualified for this recognition.

8. It was recommended by several states that in the reaccreditation of areas by the market cattle traceback method that they be given the right to include cattle tested under routine procedures to fulfill the 15 percent obligation under Part 12 of the Uniform Methods and Rules. The Committee recommends that this proposal be given thorough study and its decision placed before the organization in the 1967 meeting.

9. A preliminary draft for a proposed avian-type tuberculosis eradication program was presented to the Committee for consideration. The Committee recommends that this proposal be adopted in principle so that the first phase which has to do with educating people as to the needs for such a program could be put into effect.

10. The Committee recommends that states that do not have the authority to depopulate and pay indemnity on selected tuberculosis infected herds, attempt to acquire such authority.

11. The report by the Subcommittee on Johne's disease:
   The management of Johne's infected herds depends primarily on the stage of infection in the herd. Early infection: this refers to disease that was recently introduced into the herd and a herd test indicates that there has been little or no spread of the infection. In such herds test and immediate slaughter of reactors followed by thorough cleaning and disinfection will usually eradicate the disease in either beef or dairy cattle. Late infection: where the disease has been in the herd long enough for significant exposure of the native animals in the herd and a herd test indicates that spread of the disease has taken place, a test and slaughter program is apt to fail. Dairy herds or small purebred beef herds that have the capability of raising calves separate from their dams can use this as a method of control. Calves must be separated from their dams at birth and not be allowed to nurse. They should be raised in separate quarters on milk replacer or pasteurized milk. All equipment including foot wear and outer clothing used in the calf barn must be separate from that used in the
adult herd, or disinfected between areas. Cattle showing evidence of clinical signs of the disease should be slaughtered. Young stock must not be placed in contact with the adult herd until they calve for the first time. If young stock raised separately from the adult are sold for breeding purposes, they must be negative to the Johnin test. Commercial beef herds that cannot separate the calves from their dams present a special problem. There is at present no reliable way of controlling Johne's disease in these herds. We are in dire need of a program to offer these cattle owners that will safeguard the cattle industry without bankrupting the owner. At present research is under-way in the following areas: (1) the development of a satisfactory vaccine; (2) the development of a satisfactory diagnostic test; (3) a study of the extent of intrauterine infection. This research program should be vigorously supported.
The significance of veterinary medicine in a national emergency

A. B. Park*

Washington, D.C.

The purpose of this paper is to present a challenge to the membership of the United States Livestock Sanitary Association rather than veterinary medicine in general and not to review the role of the profession in a national emergency. A number of full length documents have been published that encompass this subject.

Anyone who has studied disaster psychology knows that after the emergency period is over the same people who have sacrificed everything for the good of the community undergo a period wherein they seek to blame someone for their misfortune. It is evident that almost without exception the blame falls on people in an official position; the mayor, the chief of police, the governor and even the President. In short, the favorite target is the government. It makes little difference who you happen to work for; local, state or federal government. Thus it is that many of us have a two-fold responsibility. Our professional responsibility while perhaps not well defined is generally understood and accepted. Our political responsibility is often not understood and may be the more important in time of a national emergency.

We may view ourselves as practitioners of the science and art of healing, prevention, control and subsequent eradication of the diseases of animals and birds but to the consumer we are a part of the merchandising of adequate supplies of nutritious meat and poultry that is safe to eat. In this context then lies the definition of a national emergency. To the Portuguese and Spanish veterinarian this would mean African Swine Fever. To the Dutch veterinarian it means Foot and Mouth Disease. To the Australian veterinarian it means Contagious Bovine Pleuropneumonia. For ourselves any of these diseases could constitute a national emergency.

What then is the challenge? There is a vast body of knowledge on the clinical course, pathology, epizootiology and diagnosis of foreign animal diseases. However we should ask ourselves whether or not our colleagues and indeed our undergraduates share our concern. Perhaps most important is the challenge that faces our teachers.

Foreign animal diseases is hardly a popular subject among most undergraduates and yet the well thought out imaginative approach used by a few of our academic institutions is well received. It is interesting that there is a concern on the part of some people that if we sufficiently motivate these men we will lose them. They are concerned that our young graduates will apply for overseas service where they can do their part in on-going eradication and research efforts. If one compares the cost of eradication in this country with the cost in the country of origin of many of these diseases it would seem to be in our interest to encourage not only our young graduates but indeed their teachers to serve an overseas tour.

In order to place this challenge in the proper context, it is important to understand the threat. An analysis has been made of the probable impact of a highly infectious contagious exotic disease. Studies have been made from time to time on the movement of livestock in the United States. Recently, however, a study was made of the movement of feeder cattle from three southwestern stockyards. This study differed from earlier ones in that an epizootiological approach was followed. When animals are moved from one assembly point to another they obviously come in contact with others, in this case other feeder cattle. We included the subsequent movement of these contact feeders. In the earlier studies only primary movements were reported but they included all species whether for slaughter, feeding or breeding. Feeder cattle only were included in this study because of the in-depth nature of the analysis.

The study indicated that the animals left the primary stockyards with one of four destinations: (1) other public stockyards; (2) livestock dealers; (3) auction markets; and (4) farms or ranches. Secondary movements of the original animals and their contacts in the first three of these destinations were followed to their final destination, that being the feedlot or ranch. Federal and state records were checked and verified by livestock inspectors at the feedlots and ranches.

An analysis of the data showed that, in the remarkably short time of three days, primary and secondary movements had extended to 170 counties in 16 states. Census data for these counties revealed that the cattle population exceeded 10 million head. It is unfortunate that owners are still being advised to "take her to the Yards" and it is worse still that many owners do this without benefit of professional consultation. An epizootic of any one of a number of foreign animal diseases could thus gain access to this fast-moving, far-reaching pipeline.

There are many members of the profession employed contractually and others in State and Federal service who have the responsibility of examining livestock moving in intrastate or interstate commerce. This brief review demonstrates the importance of their labors to the health of the livestock of this country. It is interesting to note that there have been efforts to relegate this unrewarding task to sub-professionals; whereas the singular requirement of the work is excellence in clinical observation.

A number of obvious conclusions came to light following this study. Foremost among these is the need for rapid diagnostic techniques. The challenge to our colleagues in research is not can you diagnose these diseases, but how fast can it be done. Those of us working in diagnostic laboratories should be constantly aware of the hazard of a negative diagnosis for many of our domestic diseases which are clinically and pathologically indistinguishable from these livestock plagues.

These then are our professional responsibilities. Equally important and equally serious is our political responsibility. It is clear that we need a mechanism on a national cooperative basis to stop the movement of livestock interstate and intrastate until we can find all foci of infection. One could make a very good case for the passage of laws and regulations to do this. There are however many implications of such an action. We do
not now know on a state by state basis what impact this action would have on our client, the consumer. Before such authority is sought we must know this impact. It is entirely possible and indeed probable that there are ways that this could be avoided. We must study this thoroughly and it has not been done.

Education, research and regulation are perhaps strange bedfellows, but in this particular case the need for cooperation is real. A failure on our part to accept these challenges could be damaging to us both professionally and politically.
REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES


During 1965-66, the occurrence of significant outbreaks of infectious diseases of food producing livestock and poultry in many countries give evidence of the growing need for international control. Communications from members of the Committee serving in foreign areas were discussed and note is taken of the development of animal disease problems over and above those recognized from usual information sources.

AFRICAN HORSESICKNESS

African Horsesickness was reported in Algeria in September 1965. An estimated 1,500 equidae died from the disease between September and December 1965. Diagnosis was difficult due to its similarity to other diseases. In the beginning of the epizootic, the mortality was 97 percent. Toward the end, it dropped to 90 percent. The virus is thought to have come from Chad. The virus was Type 9. Vaccine (10,000 doses) was obtained from the Razi Institute, Iran. It is interesting to compare the dates of outbreaks in Morocco and Algeria, especially when considering the optimistic views regarding control expressed in reports from Algeria. The disease spread to the southern coastal region of Morocco. The initial report (March 1966) indicated the disease had been confined to the Agadir Province. Morocco has a total population of 280,000 horses, 260,000 mules, and 1,200,000 donkeys. Losses have been estimated to be 500 horses and 1,000 mules. Another source estimated total losses at 2,500. Origin of the virus was thought to be Algeria. Vaccination, quarantine, and slaughter are being carried out.

African Horsesickness was first recognized in Tunisia in the District of Gabe in June 1966. Despite rigorous control efforts, the disease has become widespread. A second invasion appears to have occurred in the north following the course of the Mellegue and Merjeda Rivers from the Algerian borders in LeKef to Tunisia. Early in the outbreak, it was hoped the disease could be contained in the Gabe District. Now it appears that it will be necessary to vaccinate the entire equine population of the country. The total soliped population in Tunisia is estimated to be 500,000. The death loss has been estimated to be 500 animals. Since June 29, a total of 108,000 animals have been vaccinated but the supply of vaccine has been limited. It was originally anticipated that all horses and mules
would be vaccinated by August 1, 1966. Tunisia exports some 8,000 horses and 2,000 mules annually to France and Italy. This represents an export value of $2,000,000. Embargoes have been placed by both countries. Iranian vaccine (300,000 doses) has been contracted at 25 cents per dose. Nine outbreaks involving 39 animals, with two deaths, have been reported from India.

AFRICAN SWINE FEVER

Spanish researchers have demonstrated that the virus of African Swine Fever (ASF) may be harbored in *Haemotopinus suis* for more than 42 days and that this louse is capable of passing infection from pig to pig. Several *H. suis* were collected from animals dying from ASF and placed on healthy pigs which died at the end of 42 days. With the exception of leucopenia, no classical symptoms were manifested. However, 24 hours before death inappetence and a temperature of 41.2°C were noted. The possibility of latent infection being transmitted by ectoparasites dictates the need for increased consideration in their control in combating outbreaks of ASF. Outbreaks of ASF in Spain numbered 267 in 1965 compared with 427 in 1964. At the beginning of 1966, infection was reported in nine provinces.

ASF outbreaks in Portugal numbered 1,434 in 1965 as compared with 305 in 1964. The Island of Madiera recently reported an outbreak of ASF.

RINDERPEST

Rinderpest has been diagnosed for the first time in history in about 50 localities in Libya. The disease apparently gained entry through the holding pens in Tripoli where slaughter cattle from the Sudan and Romania were being held.

The annual report, "Contagious Diseases Bulletin," Indian Council of Agricultural Research, New Delhi, recorded 294 rinderpest outbreaks affecting 5,937 bovines with 2,245 deaths. Several outbreaks occurred in peninsular area of India considered free of the disease in recent years. Three outbreaks in sheep affecting 83 head with 39 deaths occurred.

An isolated outbreak of rinderpest in cattle and game animals occurred in Tanzania after four years of freedom from the disease.

Outbreaks occurred in the area of Jeddah and Mecca, Saudi Arabia, where the disease was confined and suppressed.

The regional control scheme for periodic vaccination and re-vaccination on a national scale in West African countries is proving very effective. Outbreaks in the region were much reduced, and no outbreaks occurred in several countries including the once heavily-infected Cameroun.

FOOT-AND-MOUTH DISEASE

The Indian Council of Agricultural Research reported 2,934 outbreaks involving 154,928 animals with 418 deaths.
The Union of South Africa, free of foot-and-mouth disease (FMD) since 1961, reported SAT 1 in April 1965.

An article in the newspaper "Novedades" (Mexico City) dated March 24, stated: "The possibility of foot and mouth disease outbreaks threatens livestock in the Central-American zone from Panama to Mexico, according to a warning made yesterday by the Panamanian Ministry of Agriculture. This danger comes from the decision by Colombian authorities to repopulate the Chocco region with animals from other parts of Colombia." They point out that the FMD Agreement between Colombia and the countries forming the International-Regional Animal Health Organization (OIRSA) is no longer in effect. This agreement provided for a barrier zone 30 kilometers wide on the Colombian side of the Panamanian border.

Type O which had not previously occurred in Ecuador was recorded in mid 1965.

Type O was identified in four of five infected provinces in the Philippines. Cattle were less severely affected than buffalo.

For the first time since 1960, Type O appeared in Southern Sweden in March 1966. Following slaughter of the affected animals, the country was again declared free.

In the USSR and Thrace, an A22 strain re-appeared against which conventional A vaccines were ineffectual.

**MELIOIDOSIS**

*Pseudomonas pseudomallei* is essentially a soil and water saprophyte present in a wide variety of environmental conditions in Southeast Asia. High HI titers have been detected in the sera of animals and people closely associated with paddies, and the organism has been recovered from fatal cases. In some areas, melioidosis prevents the raising of swine unless they are kept off the ground. Infected persons returning to the United States from Southeast Asia may offer a means for the possible establishment of the disease in swine.

**VESICULAR STOMATITIS**

Both the Indiana and New Jersey Types have not been identified in South America. Vesicular Stomatitis has now been reported since 1963 in Argentina, Bolivia, and Brazil.

**AVIAN DISEASES**

*Newcastle Disease*

For the first time since 1932, Newcastle disease appeared on farms in Brisbane, Australia, in February 1966, and positive serological reactions were found to be widespread.
Laryngotracheitis

*Norway* experienced laryngotracheitis for the first time in 1965. The disease has been confirmed in Greece and Japan. Previously, it had only been suspected in these countries.

**Avian Infectious Bronchitis**

A diagnosis of avian infectious bronchitis was made in Spain.

**FOOD AND VETERINARY MEDICINE**

There is an increasing awareness of the pressing demand being placed upon the United States for food by a world population which is expected to double within the next 35 years.

The United States has had significant food surplus; however, the supply of animal proteins is minimal, and stores of other commodities are rapidly declining. Two-thirds of the world's population lives in developing areas where the food supply is inadequate, and famine is approaching in several countries.

It appears that progress in animal disease control in the food deficient areas and in the less developed areas of the Americas has not been sufficient to provide an increased supply. The possibility of meeting protein requirements for a rapidly rising population with the next decade or so is indeed grim. The President's Scientific Advisory Committee's Study on World Food Supply now underway provides evidence of the seriousness of the situation, and at least one qualified expert has stated that production of all types of food must be increased by 150 percent and animal food by 200 percent by the year 2000.

Livestock diseases handicap the production of food, and prohibit the distribution of animals and animal products, thereby limiting trade between agricultural and industrial countries. This limitation is detrimental not only to their respective agricultural and industrial development; but to the well being of their populace as well.

While animal nutrition, breeding, management, and marketing are all essentials of the efficient animal production, they cannot be effective without control of the major infectious and parasitic diseases.

To fulfill its increasing responsibilities the veterinary medical profession must increase disease control efforts and intensify research by all available methods. The time factor has become so critical that more veterinarians must be trained as disease prevention specialists capable of increasing the efficiency of livestock production at home and abroad.

The American National Cattlemen's Association expressed concern that certain zoo animals might be given special consideration in the process of being imported which may endanger this country's livestock population. Others expressed similar concern for the lack of an official system of control of zoo animals other than ruminants and swine.

The Committee has suggested that the Chairman request the President to enlarge the Committee to insure a quorum at future meetings, to
enhance the expertise of the Committee, and to spread the work load involved in the proposed revision of the 1964 Foreign Animal Disease publication. Committee members will submit names of proposed candidates for such appointment.

FOREIGN ANIMAL DISEASE COMMITTEE RECOMMENDATIONS

1. The establishment of a subcommittee to study the problems that will be associated with the movement of livestock during outbreaks of foreign animal diseases. The subcommittee should include representatives from the Vesicular Disease Committee; Stockyards, Marketing and Transportation Committee; the Foreign Animal Disease Committee; Committee on Laws and Regulations; and any other committee deemed appropriate by the President.

2. USDA enter into an agreement with AID to provide veterinary assistance to organize and conduct field programs of animal disease eradication in those countries requesting assistance. The agreement should also establish a system to enable USDA to send veterinarians into areas where foreign animal diseases are occurring.

3. USDA develop regulations to control the importation of zoo animals, in addition to ruminants and swine, since all animals may be affected with ectoparasites capable of introducing and transmitting animal diseases. If approved by USLSA, the foregoing recommendation of the Committee should be brought to the attention of ARS by letter from the President of USLSA to the Administrator of ARS.
EQUINE INFECTIOUS ANEMIA
Guelph, Ontario, Canada

A great deal has been written and said about equine infectious anemia in the past two years. A recurring point in such discussions is that little progress has been made, or little information gathered, since Dreguss and Lombard published their monograph in 1954. This is not entirely true; a great deal of information has been accrued since that time and it is the purpose of this communication to discuss briefly some of these newer findings. Some of these results are from work underway in our laboratory; some are from the published literature; and still others from personal communication with workers actively engaged in the field.

DEFINITION

Equine infectious anemia is a transmissible disease of Equidae presumably due to a virus. The disease may present itself as an acute, subacute or chronic infection but horses which survive apparently remain carriers of the virus for life. The disease is named for the characteristic progressive anemia seen in infected animals. An excellent review of the clinical disease has recently been published by Hyslop.2

LABORATORY FINDINGS

Hematological Findings

Characteristically an anemia is found in the acute and subacute forms of the disease. Generally normoblasts and other immature forms of red blood cells are absent from the circulating blood but often leukocytes are seen which contain hemosiderin, these cells, siderocytes or sideroleukocytes, are suggestive but not pathognomonic of the disease. The total leukocyte count is decreased during initial attacks due mainly to a decrease in lymphocytes. During remission the count is normal.

The sedimentation rate is increased but this is merely a reflection of the reduced erythrocyte count. If the values are corrected for packed cell volume there is no significant change in the sedimentation rate.

Serum Protein Alterations

Gainer 3 has studied the alteration in serum lipoproteins and in serum lactic dehydrogenase found in the disease. He found that normal horse serum contains between 149 to 394 mg. percent (means 301 ± 97.5) lipid, whereas horses in the acute form of infectious anemia show a marked

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increase, as high as 2550 mg. percent. This increase is due to an increase in the beta-lipoprotein fraction; the alpha fraction decreases in the acutely affected horse, as low as 2.5 percent (normal mean 57 ± 7.5).

In the subacute form of the disease there is a marked change in the electrophoretic pattern of horse serum. There is an increase in the globulin fractions and a decrease in albumin. Russell, Livingston and Moore have studied the fluctuation of serum proteins during the course of the disease. They found that the albumin/globulin (A/G) ratio fluctuated with changes in the body temperature and that the degree of fluctuation was related to the clinical severity of the disease. Prior to experimental infection the A/G ratios were between 0.50 and 0.80 and decreased, to a variable degree after inoculation of virulent blood. In the acute disease the ratio decreased to between 0.30 and 0.40; in the subacute disease to 0.30 to 0.35; and in chronic carriers or horses showing one febrile attack after inoculation, the ratios approximated normal.

In addition to changes occurring in the A/G ratio, an abnormal peak was found in the region of the gamma globulin. This abnormal peak generally coincides with the onset of the febrile response and this protein is the basis of the precipitin test described by Moore, Livingston and Redmond.

Lactic Dehydrogenase

With the progression of fever lactic dehydrogenase levels rise rapidly from a normal mean of 572 ± 148 to a level of 1500 to 2000 units or higher. This increase is found in fraction five, the fraction found in liver, skin and skeletal muscle but not in the adult erythrocyte. The increase in this fraction probably reflects the amount of liver damage present in the infected animal.

Both the altered serum lipoproteins and the altered lactic dehydrogenase return to normal between fever cycles.

Virus Cultivation

For a number of years Japanese workers have been able to grow the agent responsible for this disease in cultures of horse leucocytes. The method, we have found, is a difficult one since not all donor horses yield leucocytes which show the characteristic cytopathic effect of clumping and piling-up of infected cells. In addition it is often difficult to isolate the agent directly from infected horse serum. If the serum content is increased in the tissue culture medium enhanced cytopathic effect is noted.

Recently Saurino using KB cells, has been able to recover agents from the serum of infected horses. Partial characterization of these agents has revealed the presence of protein and deoxyribonucleic acid, thus presuming that they are viral in nature. Using DEAE columns these viruses have been partially purified and characterization studies are underway. Titers in KB cells vary between 10².₅ and 10⁴.₇ TCD₅₀ per ml.

The extreme resistance of the virus to the external environment is well known, and deserves little comment here.
Diagnostic Tests

All of the diagnostic tests employed at the present time are experimental, and none of them has proved to be infallible in the diagnosis of infectious anemia at the present time. The only positive test is that of horse inoculation using methods laid down in the "Prospectus on Equine Anemia" laid down by National Organizations, including this one, and available from Dr. Ralph C. Knowles, of the United States Department of Agriculture in Washington, D.C. See pages 245-254 of this Proceedings.

Probably the most widely publicized test is that of Moore. This precipitin test, performed in tubes, depends upon the recognition of the abnormal protein peak found in the blood of infected horses at certain times, and also, apparently, produced by infected leukocytes grown \textit{in vitro}. Antiserum against this substance is produced in either sheep or rabbits and then used in the precipitin test using suspect serum as antigen.

The test, as Moore has pointed out, has several limitations. The antigen is an extremely fragile one, destroyed by heat, freezing and storage; hemolysis of the suspect serum interferes with the test; false negative results occur due to either destruction of the antigen or antigen at such a low level that it is not within the sensitivity limits of the test. Also the amount of antigen which circulates in an infected horse varies from week to week and because of this a minimum of two serum samples, taken seven days apart must be submitted from each suspect horse. Within the limits of this test any horse which gives one positive reaction to the test is presumed to be infected, regardless of the results of any other precipitin test done on the same horse.

Saurino\textsuperscript{7} is evaluating the immune adherence test in tissue culture and the indirect hemagglutination test employing human 'O' erythrocytes coated with 19S antibody from infected horses. Although the tests appear promising they have not been evaluated properly at the present time.

The diagnostic aids used by the Japanese in their slaughter policy for EIA consist of hematological examination, liver biopsy, and the number of erythrocytes along with the observation of intermittent fever and cardiac insufficiency.

If a horse has one or more of the following changes they are considered to be infected and slaughtered:

a) a siderocyte count of one or more per 10,000 leucocytes;

b) a red cell count less than five million per cubic centimeter in association with febrile attacks and cardiac insufficiency;

c) characteristic lesions found on liver biopsy.

It may be seen that the present diagnostic methods rest, at least, upon shaky foundations. If they detect a positive horse it is generally the horse in the acute or subacute phase of the disease and no test at the moment, with the exception of a properly carried horse inoculation, can detect the carrier horse who is asymptomatic and so doubly dangerous in the epizootiology of the disease.
We now enter the realm of the speculative, although a few facts and analogies are helpful in making some sense out of the pathogenesis of the progress of this disease.

We are faced with two basic problems, generally unknown to virology when dealing with such simple infections as those due to enteroviruses and myxoviruses, and even with reactivation of animal herpesviruses such as infectious bovine rhinotracheitis and equine rhinopneumonitis viruses. First of all the agent of EIA does not appear to stimulate the formation of virus neutralizing antibody, and the pathological changes in an infected horse more closely resemble those of an autoimmune disease than they do a viral infection.

A number of presumed virus infections such as plasmacytosis of mink and Riley virus infection (LDH elevating factor) of mice do not, apparently stimulate neutralizing antibody. In the case of Riley virus, Rawson, Mahy and Bendinelli have recently shown that the virus can produce a constant viremia despite the presence of neutralizing activity present in this viremic blood. They offer a solution to this problem by suggesting that a fraction of the virus is not neutralizable and so is capable of invoking persistent viremia. This may be analogous to the situation in EIA.

Autoimmune disease may be stimulated by chronic injury to normal tissue which leads to a slow but constant release of sequestered normal antigens from damaged cells. Due to sequestration, the animal would not recognize these antigens as "self" and antibody would be produced against them leading to further tissue damage.

Serum samples from rats following carbon tetrachloride administration exhibit a capacity to fix complement when incubated with antigen derived from isologous or autologous rat liver or with extracts of other rat organs. In addition circulating autologous hepatocellular antigens have also been demonstrated in rats poisoned with thioacetamide.

Complexes of antigen, from liver or elsewhere, with antibodies in excess may result in the perpetuation and accentuation of an existing hepatic lesion if such complexes are deposited in the liver. It may well be that such is the case in EIA, rather than a constant destruction of liver and other cells by the action of the virus alone.

We have become interested in this theory in the laboratory and have been able to show to date 1) the presence of circulating antibody directed against infected and normal liver by both the complement fixation test and the indirect hemagglutination-inhibition test, 2) bound anti-horse gamma globulin in tissue, demonstrable by the fluorescent antibody procedure, and 3) a positive indirect Coomb's test in infected horses.

These findings we believe are indicative of an autoimmune phenomenon in equine infectious anemia and they are being actively pursued in our laboratory, along with characterization studies on the infective agent.
REFERENCES

EQUINE INFECTIOUS ANEMIA—THE SECOND TIME AROUND
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INTRODUCTION

"Equine Infectious Anemia—The Second Time Around" was selected as the theme for this presentation not because there have been breakthroughs that will point the way towards successful control and eventual eradication but it is in spite of the lack of progress that we have made that we must create a new era in EIA. We cannot tolerate living with the condition as we have the past seventy-five years; this period must be regarded as history and we must start anew with imagination, and insight and enthusiasm. The value of the increasing horse population, the new mobility and concentration of this population, the lay use of hypodermic syringes all make the creation of such an attitude an absolute necessity. The industry owes it to itself and the public it serves. To do less would be to defile the trust that is residing with us.

First we must admit that EIA is a present problem. As this becomes a matter subject to open discussion instead of clandestine whispers, and as soon as all elements of the industry will recognize that ignoring the problem will not cure it, then and only then will the leadership be able to spark sustained investigation and research which will lead to eventual success.

While this audience needs no instruction in the known character of the disease it is worthwhile to review some facts which help to explain why EIA remains an enigma. First, no other host animal can be consistently infected, thus making the horse the only useable laboratory host. Secondly, despite many attempts to develop serological methods of testing—complement fixation, precipitin and hemagglutination—none have been developed that are consistently accurate. And third, so far as is known an animal once infected carries the potential for spread of the disease for the rest of its life. Lastly, the extreme variation in clinical and pathologic evidence make the disease most difficult to diagnose on these bases. To this time, transfer of the disease from the suspect animal to a test horse has been and continues to be the basis for sound diagnosis. And to a degree, this method is suspect unless possibility of latent infection or immunity in the test animal is excluded.

In brief, EIA is a disease of outstanding character, vigor and subtlety. Completely individual in form and distinct in manner, it has resisted the prying and probing of some of the finest scientific talents available.

This project by the Division of Animal Industry, New York State Department of Agriculture and Markets was at and in cooperation with the College of Veterinary Medicine, Cornell University, Ithaca, New York.
Following a severe outbreak of EIA interest frequently waxes, and wanes when the number of reported cases lessen. And well it may wane, for investigation of the infection has been fraught with confusion and the one sure end has been frustration.

The causative organism is a filterable virus which essentially is transmitted only by parenteral introduction. Biting insects are probably the chief vector in Nature; surgical instruments, tattoo equipment and all too commonly, hypodermic needles are the principle Manmade vectors. The fact that inhalation and ingestion are not known to be significant in the spreading of infection creates the only built-in advantage in control that we possess. While spreading appears to be slow and sporadic it must be remembered that observable spread is slow, the actual extent of infection will not be known until more adequate test methods are available.

While the disease is described as existing in acute, semi-acute, chronic and inapparent forms these terms are useful for description and cataloging only. The boundaries of the several types are vague and the form in a victim may change at anytime.

Over the past several years the New York State Division of Animal Industry has maintained what has necessarily been an interested bystander involvement in EIA. The nature of the disease made it apparently impossible to control so no active program was undertaken. As far as we were concerned the disease was unmanageable in fact as well as in theory. Our stand had been undergoing reevaluation in the light of the obvious need for protection for our rapidly growing horse population (there were an estimated 125,000 horses in New York State last year) and the great financial stake that the State had in the horse industry. (In 1965 New York State received $140,000,000 in pari-mutuel funds and fees from horse racing.)

In February of this year the report of an infected stallion at an Eastern track and the announcement of a serological precipitin test competent to detect carriers spurred a change of attitude. Actually there was one event not two, for the diagnosis of EIA in the stallion was based on a positive precipitin test.

The Texas precipitin test, developed by Doctors Moore and Livingston is reported to measure an antigen in the serum of EIA horses. The test does not detect the presence of virus. The developers state that this antigen is not present at all times; therefore the serum should be tested at least twice at a weeks interval. Negative tests may be false, positive tests are considered valid.

The Division of Animal Industry initiated a study to determine (1) if the suspect animal was positive on horse inoculation, and (2) to what extent the disease may have spread to contacts. It was assumed that through this work a further evaluation of the precipitin test would be accomplished. Dr. Moore graciously and enthusiastically agreed to perform all the precipitin tests necessary.

The second objective—to determine the extent of spread—was started by a traceback study of animals that had been in contact with the victim.
Through its movements a very imposing number of contacts had taken place, these varied from animals in the same van for a few hours to those in adjoining stalls for many days. A traceback search on an active race-horse is like drawing a criss-cross map of the country and to list contacts during its travels is to create a veritable cross section of breed registry. The opportunities for contact and spread are almost awesome. Finally, twelve animals in addition to the original stallion were selected for study. While these were regarded as contacts they may well be considered more of a random sampling.

METHODS

Twenty young Shetland-type ponies were purchased from a single farm for clinical testing. After weighing and worming and receiving permanent identification these ponies were placed in straight stalls and a ten day observation period was begun. During this period animals were temperatured in the morning and on two occasions blood samples were drawn for study. Serum for precipitin testing was obtained on these days and the serum was forwarded to Texas A & M.

Following the observation period fourteen animals received 30 mls. of blood from the thirteen previously sampled Standardbreds; two (#4 and #8) of the fourteen ponies received blood from the suspect stallion originally brought to our attention. Six ponies were maintained as controls.

The post inoculation observation period was to last 60 days. This was determined because of previous experience when we had observed incubation periods in excess of the 40 day period prescribed in the Prospectus. During the post inoculation period each animal was temperatured twice daily. Whole blood and serum samples were taken daily. Hematocrit values, total and differential counts were done daily as well as additional tests when indicated; serum samples were submitted to Texas A & M.

RESULTS

Three (#7, #13 and #19) ponies had positive precipitin tests during the preinoculation period. These three were maintained as positive precipitin controls. The other three controls were precipitin negative.

At the end of the sixty day post inoculation period four (#4, #8, #9 and #18) ponies had been diagnosed as clinically positive. Diagnosis was based on temperature patterns and alterations in hematocrit values in conjunction with clinical symptomatology. Two (#8 and #9) of the four clinically sick ponies had had precipitin positive tests. The other two (#4 and #18) were positive in additional testing after the sixty day period had been completed. (Figures 1-5).

Of the sixteen clinically negative in the post inoculation period, seven had been positive on the precipitin test, included in these seven were the three precipitin positive controls and one previously negative control (#15). A second precipitin negative control (#5) showed positive in testing.
Figure 1. Negative test. Note extreme variations of hematocrit values.

Figure 2. Test pony #4. Received blood from original suspect stallion.
Figure 3. Test pony #8. Received blood from original suspect stallion.

Figure 4. Test pony #9.
following the thirty day period. None of the control animals developed any symptoms that would suggest EIA. (Figures 6-12).

The three (#7, #13 and #19) precipitin positive controls were further evaluated: blood from each was passed to another test animal and the
Figure 7. Test pony #19. Precipitin positive control.

Figure 8. Test pony #15. Precipitin negative control initially; precipitin positive in later period.
TEST PONY #10

Figure 9. Test pony #10. Clinically negative; precipitin positive.

TEST PONY #20

Figure 10. Test pony #20. Clinically negative; precipitin positive.
TEST PONY #14

Figure 11. Test pony #14. Clinically negative; precipitin positive.

Figure 12. Precipitin test summary. Circles indicate negative tests; solid squares positive test. All animals tested twice in pre-inoculation period, daily for sixty days post inoculation and selected testing for additional fifteen days.

three controls each received blood known to contain virus. The three animals that had received the blood from the controls failed to sicken although later proved susceptible. The precipitin positive controls themselves were challenged with known virus and each sickened with EIA.

Following the post inoculation period the remainder of the clinically negative test and control animals were challenged with known virus and each proved susceptible except for one control animal, #15. This animal
had one temperature reading of 102.9 in the forty five days after inoculation and no alteration in hematocrit values. It will be referred to again later.

DISCUSSION

The clinical aspects of EIA have been well documented but our experience in both field and experimental cases reinforce the thought that the literature fails to emphasize the multiplicity and sublety of forms. In non-frank cases the diagnostician must be prepared to invest the most detailed and meticulous observation and evaluation. Not uncommonly, the most modest of symptoms displayed in a cyclic pattern will provide the suggestion that will lead to a diagnosis.

The variations that may exist are well illustrated by test ponies #4 and #8. Each received blood from the original suspect stallion but the response was completely individualistic. This pattern was repeated in other cases observed. The response of #4 is also an illustration of the extended incubation period sometime experienced—this animal would have been classed as negative under the guidelines laid down in the Prospectus.

The failure of the precipitin test to accurately detect clinical cases and the appearance of false positives indicate that the test is not competent to be used as the basis for a disease control program.

The fact that three of the thirteen animals sampled in the field were revealed as EIA carriers is interesting but obviously should not be the basis of any thesis about the percentage of infection existing in the population. It should be noted however that two of the twelve random samples had gone unrecognized and as such constituted a continuing danger to other animals.

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Following the initial work described above, other diagnosis-control aspects of the disease were explored. These were directed toward securing a greater comprehension of the disease process and a more clearly defined, rapid clinical test until that time when satisfactory in vitro methods are available. Recognizing that a stress factor is involved a form of measureable stress was sought. Bleeding on a weight-volume basis was attempted, and while originally this seemed of little use, later study has suggested that there may be an application for the idea.

Of more immediate value was the response that was observed when corticosteroid drugs were administered to animals carrying the EIA virus. Although the volume of observations is limited, the following hypothesis was constructed: Desamethasome (Azium) administered at the rate of 5 mgs. per 100# of body weight for five days along with prophylactic doses of penicillin-streptomycin will not cause symptoms that duplicate EIA in a negative animal; given the same regimen a symptomless carrier or animal in the quiescent stage will show symptoms. Test animals given this dosage at the time of inoculation will have a shorter incubation period and
a more profound response than a control animal receiving the same inoculation.

This concept was utilized in the following manner: Blood passage as dictated by the Prospectus had proven to be cumbersome and expensive of time and ponies. To facilitate the selection of test animals we administered azium-penicillin-streptomycin to ponies purchased as test subjects.

Azium is also administered to animals that are negative at the end of a sixty day test period of observation. The purpose is to aggrevate any symptoms that may have been hidden, thus providing a check on our diagnosis. If test animals remain negative after a series of injections of azium, they are challenged with known virus.

Finally, we have been interested in the effect gained when Azium is used in paired test animals. In this technique, we administer suspect blood to a test animal in the usual manner; its mate received Azium-penicillin-streptomycin the day before inoculation, the day of inoculation, and for three days thereafter.

**METHODS**

1. Desamethasome (Azium) was administered intermuscularly to twenty-three animals not known to be infected with EIA—some had been clinically negative test animals and others were animals that were purchased for future use as test animals. Desamethasome (Azium) was also given to three animals known to be infected but which were quiescent. Eighteen of the twenty-three animals not known to be infected received penicillin-streptomycin prophylactically; the three known carriers did not receive antibiotics. Desamethasome was given at the rate of 5 mg. daily/100 lbs. bodyweight for five days except in the cases of two of the known carriers (#4 and #8) which received 3 mgs/100 lbs. bodyweight for five days.

Number 4 infected with blood from the original suspect stallion had appeared normal for a period extending from post inoculation (PI) day 55-91 when the drug administration was initiated. Number 18 had been quiescent from PI52-91 when treatment commenced. Number 15 was the control animal in the original project that had later received known virus and failed to respond except for an evening temperature of 102.9 on PI 17. Treatment of #15 began on PI55.

2. Desamethasome (5 mg/100 lbs)-penicillin (400,000 u/100 lbs)-streptomycin (0.25 Gm/100 lbs) was administered to one of a pair of test animals one day before, the day of and for three days following inoculation with suspect material. The other animal received suspect material. Three pairs have completed this course of diagnosis.

**RESULTS**

1. Four of the twenty-three presumed to be normal animals displayed one or more temperature readings of 102.0 or greater in the two weeks following the last injection:
Number 3 had an AM reading of 102.7 on PI 3 and a concurrent pharyngitis. This animal had not received antibiotics.

Number 41 had a PM temperature of 102.1 on PI 1 and a PM temperature of 102.0 on PI 2. This animal received antibiotics along with the desamethasome.

Number 42. This animal's response is illustrated in Figure 13.

![Figure 13. Test pony #42.](image)

Number 45 had a PM temperature of 102.2 on PI 3; PM 102.4 on PI 4; PM 102.5 on PI 5; PM 102.3 on PI 7. Morning temperatures were normal during this period and the animal had a severe cough.

Numbers 3, 41 and 47 were not regarded as EIA infections and subsequently were shown to be susceptible to infection. Number 42 was eliminated as a test animal.

Pony #4 became febrile forty-eight hours after the cessation of desamethasome and suffered six subsequent days of fever with marked symptoms of EIA. Number 18 did not respond to the desamethasome. The response of #15 is shown in Figure 14. Note that in addition to the temperature response the packed cell volumes were strongly influenced.

2. In each of the three pairs of animals the one receiving desamethasome-antibiotics had a shorter incubation period and suffered more severely from the infection as judged by length and height of fever and alteration of packed cell volume (PCV). The pair diagrammed in Figure 15 lacks some validity because of the comparatively high temperature of the animal that received drugs (T-9) just before the date of treatment and
Inoculation. Nonetheless, it is expected that the shorter incubation time and more severe attack will characterize animals receiving this regimen.

Observations in field cases which first suggested the idea of potentiating EIA symptoms through depression of the immune response plus work under controlled conditions tend to bear out the original hypothesis that desamethasone will lower the defense levels in active, inactive and
susceptible animals. Certainly such an approach cannot be considered as a definitive diagnostic means but may be a useful tool in gaining additional knowledge of the pathogenesis of the EIA virus.

While further work is necessary, we feel confident that when desamethasome-penicillin-streptomycin is given to animals according to the above protocol those that remain symptomless may be used as test animals with as much security that they are virus-free as those negative to blood passage which have been observed for forty days. The savings in time and space is considerable. It may be that the administration of antibiotics should be continued for several days after the desamethasone has stopped to make results even more clear cut.

CONCLUSIONS

1. The precipitin test fails to accurately detect all clinical infections and yields false positive reports.

2. The use of drugs of the corticosteroid group through their depression of immune responses may serve a useful purpose in defining more accurately the pathogenesis of EIA and help in the detection of cases.

3. Proof that the original suspect stallion, and two undetected animals in the random sample were carrying EIA virus suggests a level of infection that merits further investigation.

ACKNOWLEDGMENTS

While thanks are due to many for their assistance, I am particularly grateful for the enthusiastic cooperation and generous help of Dr. Charles Rickard, Chairman of the Department of Pathology. Dr. John Lowe was most generous of his time and skill in matters of management.

Dr. Mathias Kemen, ADH-USDA was indispensable in daily management and interpretation of data.

REFERENCES


THE REGULATORY AND RESEARCH EFFORTS IN RESPONSE TO THE OCCURRENCE OF EQUINE INFECTIOUS ANEMIA AT RACETRACKS IN 1965-1966

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Equine Infectious Anemia (EIA) occurred at racetracks in the United States beginning in 1964 and extending through the early part of 1966. The disease was brought to public notice during 1965 and precipitated a series of events that had widespread effects. One of these effects was the demonstration of the rapidity and effectiveness with which regulatory veterinary medicine can swing into action in the face of a rapidly developing chaotic situation. This ability of regulatory veterinarians has been proven many times with relation to such diseases as foot and mouth disease. With this condition, the required steps are well known as the pattern has been previously set and practiced several times. However, with infectious anemia, the situation was different in that it called more for education of horsemen, racing commissioners and track owners than for the usual quarantine lines and depopulation. Panic was evident in the actions of these groups. Prompt, authoritative action was necessary to prevent further deterioration of the situation and possible crippling of the industry.

Within the space of eight days in February 1965, two meetings were held that resulted in the formulation and adoption of "A Prospectus on Equine Infectious Anemia with Guidelines." The American Association of Equine Practitioners and especially their Executive Secretary, Dr. W. O. Kester, as well as the president of the United States Livestock Sanitary Association, Dr. C. L. Campbell, are to be highly commended for their roles in mobilizing and activating the organizations and persons that resulted in this decisive action. The American Veterinary Medical Association also deserved credit for lending all necessary assistance. Various research groups, industry representatives, United States Department of Agriculture personnel, state veterinarians and other individuals connected with horses or racing contributed greatly to the overall effort.

I would like to record for you, in more or less chronological order, the sequence of events as known to me or reported by various individuals, especially the excellent report of Dr. W. O. Kester.1

Dr. Paul B. Doby, state veterinarian of Illinois, provided the following:2

"Information indicates that at least two horses were confirmed as being infected with equine infectious anemia at Chicago tracks in 1964. The horses were reported ill in September and both had raced at several tracks in other states. Four horses that had clinical symptoms of equine infectious anemia died after being moved from Illinois. A track

*Florida Department of Agriculture.

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veternarian reported in May, 1965, that there were several ill horses in a Chicago track which exhibited symptoms of equine infectious anemia. This was largely confined to horses in one stable. The condition proved to be equine infectious anemia. Investigation of this stable indicated horses had raced at five tracks including California, Arizona, Mexico, Kentucky, and Arkansas, prior to entering Illinois. Before the condition was diagnosed, three of the affected horses had been moved to Kentucky to a farm operated by the trainer."

The tables on the following page illustrate a breakdown of breeds, numbers, and dates of affected animals during 1965 and 1966.

These and subsequent events led to two strong resolutions supporting regulatory efforts that were passed by the American Association of Equine Practitioners during their convention in Miami Beach, Florida early in December, 1965. These resolutions state as follows:

"RESOLUTION - Equine Infectious Anemia Research

WHEREAS, it is recognized by the American Association of Equine Practitioners that the two diseases, Equine Piroplasmosis and Equine Infectious Anemia, obviously impose a health and economic threat to catastrophic magnitude to the total horse industry in America.

WHEREAS, experience has amply proven that diseases with the characteristics of Piroplasmosis and Infectious Anemia can be controlled only on the national level by a nationally directed and coordinated program.

THEREFORE BE IT RESOLVED that the American Association of Equine Practitioners urges the Secretary of the United States Department of Agriculture to immediately formulate and implement a research and regulatory program adequate in scope to control and eliminate these two devastating diseases from our country."

RESOLUTION - Equine Infectious Anemia Control

WHEREAS, Equine Infectious Anemia, "swamp fever"—devastating disease of equines—is becoming widespread throughout the United States and is causing serious financial loss to the horse industry.

WHEREAS, outbreaks of the disease have occurred during the past season at race tracks and other horse population centers in the States, from the far north and west to the midwest and south.

WHEREAS, when such outbreaks do occur at race tracks, or comparable institutions, responsible officials find it expedient and necessary to order removal from the premises of all horses suspected of having, or carrying the disease.

WHEREAS, when such suspect horses are ordered from, or leave a race track, or other establishment, they may, and often must, scatter to various farms and stables, thus further enhancing opportunity for spread of the disease.

THEREFORE BE IT RESOLVED that the Department of Agriculture of the State or States involved, which is the agency ultimately responsible for animal disease regulation and control within the State, be urged to
Equine Infectious Anemia in Illinois

1965

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Summary

Total Suspects for 1965 ............ 49 (35 T., 12 S., & 2 Q.)
Confirmed cases (positive to precipitin or horse inoculation or died following clinical symptoms) ............ 33
Dead ........................................ 27
Alive ........................................ 22
Total Quarantined ................... 14
Remaining Under Quarantine
(12/31/65) ................................. 6
Negative on Whole Blood Test ..... 1

T = Thoroughbred; S = Standardbred; Q = Quarterhorse

Equine Infectious Anemia in Illinois

1966

<table>
<thead>
<tr>
<th>Period</th>
<th>Suspects that Died</th>
<th>Suspects Quar. Positive to Precipitin Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3 (2 S. &amp; 1 T.)</td>
<td>1 (S.)</td>
</tr>
<tr>
<td>February</td>
<td>8 (7 S. &amp; 1 Q.)</td>
<td>2 (S.)</td>
</tr>
<tr>
<td>March</td>
<td>7 (5 S. &amp; 2 T.)</td>
<td>5 (S.)</td>
</tr>
<tr>
<td>April</td>
<td>8 (S.)</td>
<td>1 (Q.)</td>
</tr>
<tr>
<td>May</td>
<td>1 (T.)</td>
<td>None</td>
</tr>
<tr>
<td>June</td>
<td>15 (9 T., 5 S., &amp; 1 SP)</td>
<td>4 (T.)</td>
</tr>
<tr>
<td>July</td>
<td>4 (S.)</td>
<td>2 (S.)</td>
</tr>
<tr>
<td>August</td>
<td>4 (3 S. &amp; 1 Q.)</td>
<td>1 (Q.)</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>50</strong></td>
<td><strong>16</strong></td>
</tr>
</tbody>
</table>

Summary

Total Suspects as of Aug. 31, 1966 .................. 50 (34 S., 13 T., 2 Q., 1 SP)
Confirmed by Precipitin Test or Horse Inoculation .......................... Pending Results
Dead ........................................ 16
Alive ........................................ 33
Total Quarantined in 1966 ........... 11
Total Alive Under Quarantine for 1965 and 1966 ........... 10

Note: This is the total as of Aug. 31, 1966.

T = Thoroughbred; S = Standardbred; Q = Quarterhorse; S = Shetland Pony
take all effective means of control and designate and provide a suitable isolation stable area, where horses suspected of having the disease may be safely stabled by their owners until adequate, acceptable, diagnostic tests have been performed and the animal declared disease-free and released by competent authority, or otherwise safely disposed of.

AND BE IT FURTHER RESOLVED that the American Association of Equine Practitioners urges responsible officials at all race tracks and other horse population centers to promptly notify appropriate State Animal Disease Regulatory Officials of any suspected disease outbreaks and further assist in formulation and coordination of suitable procedures for isolation and control to the end that further disease spread is prevented and financial losses to owners of involved horses are held to an absolute minimum."

These resolutions were widely publicized in *The Blood Horse* and *The Thoroughbred Record* and stimulated demands for action by industry people. Shortly thereafter, certain state racing commissions promulgated regulations aimed at controlling the disease or at preventing affected animals from entering tracks in their respective states. Some of these regulations were conceived without adequate professional assistance and were both unworkable and ineffectual. They were influential in the writing of the "prospectus and guidelines" referred to above.

Additionally, the disease was receiving very thorough coverage wherever it appeared or was reported to have appeared. Sixteen articles referring to the disease appeared in *The Blood Horse* between November 27, 1965 and May 14, 1966.³

At this point, toward the end of January 1966, it was obvious that action by responsible, informed people was essential. On February 8, 1966, the first meeting was held in the Washington offices of the American Veterinary Medical Association, resulting in the prospectus on equine infectious anemia with guidelines. During the next week in Atlanta, Georgia, this document was further developed and refined and presented to an emergency meeting of state veterinarians involved with control of the disease. After revision, it was adopted by this group which included over half of the country's state veterinarians. On February 22, 1966, these guidelines were presented to a meeting in Boca Raton, Florida called by the Thoroughbred Owners and Breeders Association for the purpose of hearing research proposals by various groups relative to infectious anemia. The next week the guidelines were adopted by the National Association of State Racing Commissioners and the Horseman's Benevolent and Protective Association, who gave their endorsements and did what they could to implement the guidelines as set forth.

During this time, research groups had also become concerned and held meetings in an effort to learn the *status quo* of the disease and directions research should take. The first meeting was called by the Grayson Foundation in Lexington, Kentucky, on February 17. Research completed by the participants was reviewed and future plans and possibly productive avenues of research were discussed.
W. L. SIPPEL

The meeting in Boca Raton, Florida, referred to above, was partly concerned with the same subjects. On March 7, 8, and 9, a conference was held at Texas A & M University, College Station, Texas, to demonstrate the precipitin test and to further explore other possible diagnostic tests.

On March 24, 1966, the House Appropriations Sub-Committee for Agriculture met and heard representatives requesting funds for research on equine infectious anemia. This meeting was notable due to the fact that there were present representatives of nearly all organized equine associations and all veterinary organizations concerned with horses. This meeting resulted in the recommendation by the committee for the appropriation of $200,000 for this work, $100,000 each for research and for regulatory work. The Senate Sub-Committee met in April and increased the request for research funds to $200,000.

The President signed the bill in September, 1966, at which time the funds for regulatory work were reduced to $75,000 and made contingent upon further justification of the need for the funds. This amount was ultimately approved. The $200,000 for research will be divided half for the National Animal Disease Laboratory and half to be divided among Texas A & M University, Washington State University and Louisiana State University.

Again, the United States Livestock Sanitary Association, the American Association of Equine Practitioners, and the American Veterinary Medical Association, took the lead in presenting to the Congressional Committees the case for regulatory and research needs on this condition. They were ably supported by the various horse organizations referred to above.

During July of 1966, an international conference on infectious diseases of equines was held in Stresa, Italy, supported by the Grayson Foundation and the Unione Nazionale Per L'Incremento Delle Razze Equine. This congress adopted the following resolution relative to equine infectious anemia:

The Conference considering:

1. that EIA is usually transmitted through direct or indirect inoculation of the virus and not through feeding or contact between animals;
2. that eradication of the disease can only be obtained by elimination of the carriers of the virus;
3. that no biological method of experimental diagnosis except inoculation gives nowadays completely satisfactory result;

recommends:

1. that the diagnosis can be based on:
   a) clinical signs and particularly variations of temperature
   b) hematology and particularly
      - sedimentation rate of the globules (RBC's)
      - volume of the globules (RBC) (Hematocrit)
      - albumin/globulin ratio
      - lactic dehydrogenase investigations and determination of lipoproteins
      - sideroleucocytes count
c) as a complement to the preceding tests: precipitin test, and, or, liver biopsy with specific lesions;
d) experimental inoculation of another horse if necessary;

2. that the prophylaxis should be based on:
a) isolation of suspect animals;
b) slaughtering of carriers when proven by horse inoculation because it is the only method we can use absolutely;
c) disinfection and destruction of blood sucking insects;
d) the use of disposable syringes and needles for any therapy (one syringe, one needle per horse);

is fully aware:

1. of the importance of the research being carried out in different laboratories to obtain a specific method of biological diagnosis and wishes that these works should be carried on;
2. of the interest of further studies in the pathogenesis of the infectious process;

wishes: for a good realization of this program:

1. that international sanitary regulations about this disease would be standardized with a permanent veterinary survey of racetracks;
2. that very important monetary facilities would be given to the workers by private or public organizations;
3. that other conferences on this subject should be organized in the future;
4. that until the next conference a system of liaison and very quick information be organized between everybody interested in the problems of the horse diseases."

Largely as a result of the wide publicity given the prospectus and guidelines and distribution of information on the spread of infectious anemia, the disease at the racetracks ceased to be an acute problem.

This is a fine illustration of the capabilities of regulatory veterinary medicine in the face of an urgent situation. Within the short space of one week, two meetings were held during which the methods of procedure to control this disease were formulated and approved. Within another week, these were presented to important segments of the industry and accepted by them. As a matter of fact, too good a job might have been done, for, as a result of activation of these recommendations, new cases ceased to appear and interest in the problem has dropped to the point where it has again become difficult to obtain funds from industry sources to support needed research on the disease.

The prospectus and guidelines are appended.
REFERENCES

3. Articles as follows from *The Blood-Horse*:


A PROSPECTUS ON EQUINE INFECTIOUS ANEMIA
WITH GUIDELINES

The following prospectus and guidelines for controlling the spread of Equine Infectious Anemia particularly at America's horse racing and training tracks were formulated by representatives of the following national organizations:

- American Association of Equine Practitioners
- American Quarter Horse Association
- American Veterinary Medical Association
- National Association of State Racing Commissioners
- Thoroughbred Owners and Breeders Association
- Thoroughbred Racing Association
- United States Department of Agriculture
- United States Livestock Sanitary Association
- United States Trotting Horse Association

A great deal of time and effort has been spent in evaluating the disease itself, diagnostic tests, pertinent regulatory laws and rules, current research and other factors in coordinating and developing sound ideas to serve as a basis for a national unified program of immediate control aimed at eventual elimination of Infectious Anemia.

While it is obvious that the tools at hand are less than perfect and much more needs to be known about the disease, its diagnosis and methods of controlling it—the urgency of the current situation requires the practical application of the best methods now available in order to minimize losses with the least disruption and cost to the horse industry.

Inasmuch as the majority of the nation's State Veterinarians have thoroughly reviewed, approved and endorsed the material and methods set forth herein, it is fervently hoped that the remaining states will also accept them in order to establish nationwide uniform procedures which will negate the necessity for invoking unduly stringent regulations, particularly in the field of interstate movement, which would likely prove disastrous to our horse industry. With cooperative measures between the states as is contemplated herein, it is felt that immediate outbreaks can be effectively contained until an anticipated crash research program can lead us to eradication of Equine Infectious Anemia.

1. DEFINITIONS FOR THE PURPOSE OF THIS REPORT

A. Equine Infectious Anemia - a widely spread virus-caused disease of the equine family which is infectious in nature and spread by injudicious use of hypodermic needles and other instruments as well as by insect vectors, and which may be acute, subacute or chronic in form.

B. State Veterinarian - the chief livestock regulatory official—usually an employee of the State Department of Agriculture or State Livestock
Sanitary Board and responsible for the control and eradication of animal disease within his state.

C. Commission Veterinarian - one employed by the State Racing Commission or Board for the purpose of advising and assisting with veterinary matters pertaining to the Commission and to direct the veterinary functions and activities of the Commission.

D. Advisory Committee - a group of consultants composed of practicing, regulatory or other veterinarians who may be appointed by the State Veterinarian to assist him in an advisory capacity in dealing with Equine Infectious Anemia.

E. Precipitin Test - the serological test for Equine Infectious Anemia as currently performed on horse serum at Texas A & M University.

F. Horse Inoculation Test - a test whereby 200 cc of blood is transferred from a suspect horse to a susceptible test horse under the direction of a State Veterinarian following the protocol in Appendix #1.

G. Suspect Horse - a horse showing no clinical manifestations of Equine Infectious Anemia that has given a positive reaction to the precipitin test. (Status of the disease in the horse may be further determined by horse inoculation test).

H. Infected (Positive) Horse - a horse that shows clinical manifestations of Equine Infectious Anemia and positive precipitin test reaction until proven negative to an official horse inoculation test.

2. INFORMATION ACCEPTED AS FACT PERTAINING TO EQUINE INFECTIOUS ANEMIA

A. Responsibility for Control
   (1) The individual State Department of Agriculture or Livestock Sanitary Board is the sole agency legally responsible for the control of Equine Infectious Anemia within the respective states.
   (2) The United States Department of Agriculture has certain regulatory jurisdiction over horses entering the United States or known infected animals being moved from one state to another.

B. Health Certificates
   (1) A health certificate is current and valid only at the time a horse is physically examined and the certificate issued.
   (2) A Health certificate at no time assures that a horse is not a carrier of the virus or has not been exposed to the disease.

C. The Precipitin Test*
   (1) Is a test for an antigen produced in the blood by the action of equine infectious anemia virus. It is not a test for the virus itself.

*It should be noted that Texas A & M will not have the capacity to do the anticipated volume of testing until industry provides the funds to expand existing research facilities into a laboratory capable of carrying on a large scale testing program and continuing necessary research.
(2) This antigen (which is the object or basis of the test) is fragile and easily affected by heat, chilling, freezing and storage. Consequently, it is not considered an ideal test.

(3) False negative findings may be expected due to: (a) destruction of the antigen in the blood sample caused by improper handling; (b) blood sample being drawn from a carrier horse at a time when the antigen was absent or too low to detect.

(4) The presence of the antigen in the blood serum is dependent upon the degree of activity of the virus in the horse. This activity is known to vary from week to week.

(5) A minimum of two blood samples seven days apart must be taken from each horse.

(6) A horse that has given one positive reaction to the test is presumed to be infected and a carrier of the virus for life irrespective of any previous or subsequent negative reaction to the test. (Texas A & M reports 35 controlled horse inoculation tests confirming this presumption.)

NOTE: Refer to items 1 G and 1 H on page 246.

D. The Virus

(1) The virus is transmitted from a carrier horse to a susceptible one primarily through transfer of minute particles of blood by biting insects and contaminated instruments such as hypodermic needles, tattoo needles, saliva collecting equipment and other articles or instruments which may penetrate or severely abrade the skin or mucous membranes.

(2) The virus has many unusual characteristics which make it most difficult to study and manage.

(3) The virus is not destroyed by the usual antiseptic and disinfecting solutions. It will withstand boiling heat of less than 15 minutes duration.

E. The Disease

(1) Is widely spread throughout the country at ranches and farms and more recently at race tracks.

(2) Clinically it resembles many other diseases and it is impossible to make a differential diagnosis without laboratory tests.

F. Spread of the Disease

(1) It is believed that the hypodermic needle in the hands of horse handlers is the biggest single cause for spread of the disease at race tracks.

(2) Biting insects may be the biggest factor for its spread at ranches and farms.

(3) A horse carrying the virus is of slight danger to horses stabled around it, providing: (a) biting insects are eliminated; (b) no instruments used on the carrier horse are used without sterilization on other horses; and (c) good daily sanitary practices are followed.
C. Control Measures
(1) Control depends upon the identification and eventual elimination of infected horses. This must be done in the most practicable and least costly manner.
(2) There is urgent need for a nationally coordinated control effort, along with a centralized comprehensive reporting and analyses of all cases and all tests.
(3) The suggested plans and procedures evolved in this report provide the framework for an effective control and research program.
(4) Success of any program will depend upon the cooperation and support extended by individual horsemen as well as all facets of the industry.

3. AREAS OF RESPONSIBILITIES AND FUNCTIONS
A. Responsibility for a central headquarters office for the purpose of (1) collecting and disseminating all pertinent information and (2) coordinating and analyzing all test and control procedures on a nationwide basis has been assigned to:

   Dr. Ralph C. Knowles  
   Chief Staff Officer  
   Animal Health Division  
   Agricultural Research Service  
   United States Department of Agriculture  
   Washington, D.C.  
   Headquarters Office Telephone: Area Code - 202; 388-8637

B. State Veterinarian or Chief State Livestock Regulatory Official (State Department of Agriculture or Livestock Sanitary Board)  
(1) Responsible for the control of contagious diseases of livestock within his state including disease among horses at race tracks.
(2) May appoint an "Advisory Committee" from practicing or other regulatory veterinarians including the Commission Veterinarian to assist him in an advisory capacity in case of a suspected outbreak of Infectious Anemia at any race track.
(3) Should have available to him an isolation area including screened stalls at or near each track suitable for isolating suspect horses (isolation area to be provided and maintained by track management under sanitary and isolation requirements prescribed by the State Veterinarian).
(4) In case of a suspected outbreak will, with the assistance of his advisory committee determine which horses will be subjected to the precipitin test. Will recommend which horses should submit to the animal inoculation test.
(5) Will be responsible for conducting all animal inoculation tests performed in his state as prescribed in Appendix #1.
(6) Will report the results of each test on each horse to the United States Department of Agriculture Central Office.
(7) Will maintain a list of the names of all suspect and infected horses.
(8) Will prescribe and enforce necessary sanitary rules and isolation rules at race tracks and elsewhere.
(9) Will declare Equine Infectious Anemia as a required reportable disease in states where such is not already the case so that all cases will be reported and recorded in each state.

C. State Racing Commissions and Commission Veterinarians
(1) Should see that sanitary and other protective measures prescribed by the State Veterinarian are enforced at race tracks.
(2) Should insure that tattoo instruments and saliva collecting equipment are adequately sterilized under the supervision of the Commission Veterinarian (autoclave 15 minutes at 15 pounds or thoroughly wash clean and boil for 15 minutes prior to being used on any horse).
(3) Should enforce rules preventing the use of hypodermic syringes and needles on horses by other than veterinarians licensed to practice at the track.
(4) Should require provision and operation of adequate isolation facilities acceptable to the State Veterinarian.
(5) The Commission Veterinarian should serve on the State Veterinarian's advisory committee and will promptly report all cases of reportable disease suspected at the tracks.
(6) Should assist and support an accelerated national research program to combat Equine Infectious Anemia and Equine Piroplasmosis.
(7) Should require temperatures of all horses stabled at tracks to be taken and recorded each morning and evening and such record made available, when required, to State Veterinarians. Any abnormal temperature (102°F and above) should be reported to the Commission Veterinarian.

D. Race Track Management
(1) Should institute and carry out at all times the sanitary and preventive measures outlined in Appendix #2.
(2) Should provide and maintain screened isolation facilities adequate to meet the needs of and be acceptable to the State Veterinarian.

E. Owners, Breeders and Trainers
(1) Should understand that the disease is usually spread by the transfer of blood even in the most minute quantity from an infected horse to a susceptible horse.
(2) Should realize that perhaps more horses become infected at race tracks by means of contaminated hypodermic needles in hands of horse handlers than through all other sources combined.
(3) Should realize that a perfectly normal appearing healthy horse can be a carrier of the virus for years without suspicion.
(4) Should realize that if a hypodermic needle is used on a carrier horse and later on a susceptible horse the second horse is very apt to come down with the disease.

(5) Should follow the sanitary and precautionary measures outlined in Appendix #2.

F. Practicing Veterinarians

(1) Will immediately report any horse suspected of being infected with Infectious Anemia to the State Veterinarian. If the horse is stabled at a race track he will simultaneously report it to the Commission Veterinarian.

(2) Should continue the practice of using disposable hypodermic needles and syringes (one needle - one horse).

(3) Offsize needles and other surgical and medical equipment which must be reused should be sterilized either by thorough cleaning and boiling for 15 minutes or autoclave 15 minutes at 15 pounds.

(4) It should be noted that the virus will survive boiling heat of less than 15 minutes duration and the usual sterilization solutions.

4. PROCEDURE IN EVENT OF SUSPECTED OUTBREAK

A. Any practicing or other veterinarian suspecting a horse at a track of being affected with Infectious Anemia because of either clinical signs or tests will promptly report the case to: (1) the State Veterinarian; and (2) the Commission Veterinarian.

B. The State Veterinarian and his advisory committee will then determine which horses will be subjected to the precipitin test. Contributing factors, as well as the amount of testing required, may vary from track to track; however, such determinations should be so practicably encompassing as to afford the necessary protection to the health of other horses stabled there.

C. The blood sample for the precipitin test will be drawn from the horses by a veterinarian authorized by the State Veterinarian and submitted to Texas A & M following the procedure outlined in Appendix #3.

D. Horses suspected of being infected because of a positive reaction to the precipitin test but showing no clinical manifestations of the disease (as determined by the State Veterinarian and his advisory committee) may remain at race tracks under normal working conditions under the supervision of the State Veterinarian who will prescribe and enforce appropriate safeguards as outlined in Appendix #4.

(1) It is recommended that such suspect horses be subjected to the horse inoculation test (as outlined in Appendix #1) to confirm the diagnosis in all instances possible and practicable.

E. Horses suspected of being infected because of clinical signs (as determined by the State Veterinarian and his advisory committee) will be moved into a suitable screened isolation area (provided by track management), subjected to such testing as may be required,
and maintained at the owner's expense under the regulatory sanitary supervision of the State Veterinarian until released by him.

(1) Horses determined to be infected (positive) by virtue of a positive precipitin test and clinical manifestations of the disease shall be held under state quarantine until proven negative to an official horse inoculation test (procedure outlined in Appendix #1). Such quarantine will be effected at a location approved by the State Veterinarian in either the state in which the diagnosis was made, or in the state from which the horse originated—and to which it may be returned under special permit from its chief livestock sanitary official if the animal is not at the time of its return manifesting disease symptoms.

F. A horse which has been proven positive to an official horse inoculation test shall remain under permanent state quarantine.

G. Suspect horses may be transported into other states for racing, training or other purposes, but the accompanying health certificate must attest to such "suspect" status so that proper control measures will be instituted in the state of destination.

5. RESEARCH OBJECTIVES

A. To establish the incidence of Equine Infectious Anemia in the United States.
B. To develop a practical, definitive diagnostic test.
C. To further knowledge of the epidemiology and pathogenesis of the disease upon which to base improved sanitary controls.
D. To further propagate, purify and characterize the virus.
E. To study the immune mechanisms involved as a step toward vaccine development.
F. To fully evaluate the existing precipitin test as rapidly as possible.

It is noted that facilities are available and limited research is underway in Texas and Florida.

It is noted that excellent facilities and research personnel exceptionally well qualified to work on this disease exist at the United States Department of Agriculture, National Animal Disease Laboratory, Ames, Iowa, but that they are not engaged in Equine Infectious Anemia research.

It is urged that these and other institutions with an Infectious Anemia research capability be given sustaining support.

APPENDIX #1

INSTRUCTIONS AND PROTOCOL FOR HORSE INOCULATION TESTS

1. Blood from Suspected Horse:

Telephone arrangements to be made with agency conducting horse inoculation tests prior to bleeding for each horse bled. Do not store test blood. Serum samples for precipitin tests to be collected at time blood is collected. If no previous precipitin tests have been made, one week later collect a second sample for precipitin testing.
When arrangements for horse inoculation tests have been made, collect 500 ml. of blood in an ACD bottle. Ship on wet ice by air express. Notify testing agency of airline, flight number, and time of arrival of shipment.

2. Test Horses:

Test horses are to be two years old or over and identified by hot iron hoof brand. These horses are to be bought in an area of the country where the disease is not believed to exist and purchased subject to passing two negative precipitin tests for EIA drawn one week apart and to a negative test for Equine Piroplasmosis (CF or gel precipitin). When delivered, they are to be placed in and kept in screened stalls for blood cross transfuring in groups of 3. 100 ml. of blood from Horse #1 is injected intravenously into Horse #2, 100 ml. of blood from Horse #2 is injected into Horse #3, and 100 ml. of blood from Horse #3 is injected into Horse #1. These horses are to be temperatured twice daily for 40 days and these readings recorded. Weekly hematocrit and sedimentation rate determinations are to be made. Blood smears are to be made for three consecutive days on and following the occurrence of any temperature over 102°F. If no fever reaction follows inoculation, the horses are considered susceptible and suitable test animals.

3. Procedure for Testing Suspect's Blood:

Inoculate 200 ml. of suspected horse's blood intravenously into a proven susceptible horse. The twice daily temperature for 40 days, weekly hematocrit and sedimentation rate (read at 10 and 20 minutes—normals: 10 minutes—two to 12 percent, 20 minutes—15-38 percent) determinations and three consecutive daily blood smears at time of fever (over 102°F.) are to be performed as outlined under Section 2.

If negative to fever and other clinical or laboratory signs after 40 days, this test horse will be challenged with known EIA infectious material, such as 200 ml. of infected blood or known positive lyophilized blood. Should it then exhibit temperature of 102°F. or above and clinical signs of EIA, the suspect horse will be considered negative. (In this event, and on future horse inoculation tests utilizing the other two test horses, it will be unnecessary to challenge them to prove their susceptibility if by the end of the test they have exhibited no abnormal temperatures, hematocrits, sedimentation rates or clinical signs of EIA.)

4. Information on Suspect (Donor) Horses to be Furnished Testing Agency:

Foaling date and place.
History of movements as far back as possible.
Location and date when first affected, with subsequent history.
Number of horses affected in same stable.
Same trainer and/or owner?
Precipitin and other laboratory test results.
Clinical history.
RECOMMENDATIONS FOR HANDLING HORSES AT RACE TRACKS AND OTHER LOCATIONS TO AID IN PREVENTION AND CONTROL OF EQUINE INFECTIOUS ANEMIA

1. Maintain at all times systematic and effective insect control, especially against flies and mosquitoes. Maintain stables and the immediate surrounding area in good sanitary condition at all times. This includes prompt disposal of manure and other refuse and good drainage to prevent vector multiplication.

2. Restrict the use of hypodermic syringes and other veterinary instruments to authorized veterinarians.

3. Prevent common use of any equipment such as bridles, bits, harness, curry combs, etc., that may produce skin abrasions or absorb body secretions or excretions.

4. Clean and sterilize all types of instruments used on horses including surgical, tattooing, dental, and similar items by boiling for 15 minutes, before use on each animal.

5. Use separate sterile equipment on each animal when collecting material for the saliva or other tests.

6. Frequently clean and disinfect paddocks, starting gates, and other equipment subject to contact by different animals. Use two percent trisodium phosphate to clean, and disinfect with sodium orthophenylphenate (one pound to 12 gallons of water used at 120°F or higher).

7. Require incoming horses to have veterinary health examination and certificate (including temperature recording) within 10 days prior to arrival.

8. Stable horses in individual box stalls with separate feeding and watering facilities. Daily morning recorded temperatures as well as evening recorded temperatures of each animal are desirable. All horses should be subjected to careful examination by the official track veterinarian at his discretion.

9. Stable all horses presented as race entries so as to be under the health supervision of the official track veterinarian.

10. Immediately report to the State Veterinarian all horses suspected of having an infectious, contagious or communicable disease.

PROCEDURE FOR SUBMITTING SAMPLES FOR PRECIPITIN TESTS

1. Two samples of blood are to be collected from each horse seven days apart.

2. Collect 10 cc of whole blood aseptically. Let stand at room temperature and clot. (Do not refrigerate.)

3. Centrifuge sample and collect serum in a sterile rubber stoppered vial. (If sample is hemolized, discard and collect another.)

4. Refrigerate serum immediately.
5. Ship (air mail) under refrigeration (do not freeze) to Department of Microbiology, College of Veterinary Medicine, Texas A & M University, College Station, Texas

6. Samples should be in the laboratory no later than three days after collection.

7. It should be noted that the non viral protein antigen which is the object of the test is quite fragile and easily affected by heating, freezing and storage; consequently, all samples should be collected and handled carefully as indicated above, otherwise false negative reactions may result.

APPENDIX #4

MANAGEMENT OF SUSPECT HORSES NOT SHOWING CLINICAL SIGNS OF THE DISEASE

The following are conditions and regulations under which suspect horses may be allowed to work and race under approximately normal conditions:

(1) Such horses shall be maintained under conditions of adequate insect vector control.

(2) All grooming and other horse equipment used on a suspect horse will be confined to that horse and used on no other until sterilized as prescribed and controlled by the State Veterinarian.

(3) Suspect horses' temperatures will be taken and recorded each morning and each evening and reported to the State Veterinarian.

(4) Protective measures and methods by which the disease spreads should be thoroughly explained to all involved horse handlers by the State Veterinarian.
EQUINE INFECTIOUS ANEMIA—A DIAGNOSTIC PROBLEM

R. W. Moore, D.V.M., M.S., H. E. Redmond, D.V.M.,
and D. H. Lewis, B.A., M.A.
College Station, Texas

INTRODUCTION

Several serologic procedures have been proposed for equine infectious anemia. Dreguss & Lombard\(^1\) have adequately reviewed all of these proposed tests. This report will consider the newly described precipitin test, its advantages, disadvantages and mechanism of action. The report will also describe some problems associated with horse inoculation as the final criteria of infection. A similarity exists between the precipitin test and Dreguss & Lombard's hemagglutination test and these mechanisms will also be described.

PRECIPITIN TEST

The precipitin test is performed by the addition of 0.2 ml. of the suspect horse serum to a 6 x 50 mm. tube. An antiserum made in rabbits\(^3\) or sheep\(^4\) is carefully layered over the horse serum. This is one of the inherent disadvantages to the test in that this test measures a circulating antigen instead of antibody. Since it has been shown that the antigen fluctuates in the various stages of the disease, a given horse may be positive and a few days later become negative. At the present time, we are only using antibody made in sheep; so only this procedure will be described.

Cell Culture Procedure

Whole blood from a horse proven to be free of Equine Infectious Anemia (EIA) is obtained using heparin as an anticoagulant. After collection the blood is placed in previously chilled, sterilized graduate cylinders. The graduate cylinder containing the heparinized blood is then placed in a 40°F refrigerator and the erythrocytes allowed to settle in the cold for one hour. The plasma, free of RBC's, is removed from the upper part of the cylinder with a 19 gauge five inch needle and syringe. The leucocytes are still suspended in the plasma and have not formed a buffy coat in this period of time.

The plasma containing the leucocytes is diluted one part to four parts in the following maintenance medium: Medium 199 containing 0.5 percent

From the Texas Agricultural Experiment Station, College of Veterinary Medicine, Texas A & M University, College Station, Texas. Dr. Moore is Associate Professor, Dr. Redmond, a Professor and Mr. Lewis, a graduate assistant in the Department of Veterinary Microbiology. This research was supported in part by the American Quarter Horse Association through Morris Animal Foundation.
lactalbumin hydrolysate, 200 units/ml penicillin and 100 ug/ml of streptomycin. The diluted plasma is dispensed in 15 ml amounts into sterilized milk dilution bottles. The bottles are stoppered with rubber stoppers and placed in a 37°C incubator on one of the flat sides of the bottle. After 24 hours the cells have formed a layer on the glass surface and the plasma-maintenance medium supernatant is replaced with only medium 199 plus the above additives.

In four to five days the cultures are ready for inoculation with the EIA virus. Initially the source of the virus was blood from a naturally infected carrier horse. We now use high cell culture passage virus as the inoculum. One ml. of the previous cell culture passage is added to the cell sheet after removing the maintenance medium and more maintenance medium is then added to the culture. Ten to 12 days after inoculation, the cells become granular and tend to migrate toward centers on the glass surface. At this time the cultures are frozen.

Reagent Production

Sheep approximately nine months of age are used to produce antibody for the precipitin test. The sheep are first tested against known positive and negative horse serums before inoculation. It is necessary to maintain the sheep on hay and water because grain will cause the sheep serum to be slightly cloudy. The cloudy serum moving through the horse serum will form a line which makes reading difficult. When the sheep have been determined to be negative to both types of horses, they may then be used for the production of hyperimmunized serum.

The above described cell cultures are thawed, centrifuged at 2000 x gravity to remove the cells and 10-15 ml. amounts of the supernatent are inoculated intraveneously into the sheep at three to four day intervals. After 10 inoculations the titer in the sheep is determined on known infected horse serums and if the sheep react satisfactorily they are exsanguinated. The serum from each sheep is checked with known positive and negative horse serum samples. Each sheep serum that reacts satisfactorily to the known positive and negative samples is then pooled into one lot. The pooled lot is divided into five ml. amounts and frozen at -20°F and remains in storage until ready for use.

Reading and Interpretation

After the sheep serums has been layered over the horse serum, the test is observed at hourly intervals for four hours. A line of precipitate forming at the interface of the two serum samples is considered to be positive. The test is difficult to read but with a few precautions, many of the difficulties can be overcome. These precautions are: (1) Read only precipitate and not dense line at the interface. (2) Fasting of the sheep before bleeding gives a reagent that is clearer, and therefore it is easier to see the precipitate. (3) Reading at hourly intervals helps because only one dilution is run and optimum antigen-antibody ratios may occur at one hour but not exist at four hours and also the reverse may be true. (4) Agar gel has been tried but the protein does not move in agar so the precipitate
forms in the horse serum only. (5) Some pony, jack and jenny serums react with the sheep serum forming a cloudy band at the interface which makes reading and interpretation almost impossible. This is especially true in the case of blood samples that do not release serum and excessive centrifugation is preformed on the sample. This problem has not occurred with horse samples. (6) Remember that the horse serum contains the antigen and antigens are not as stable as antibody to anything that denatures protein. Therefore preservatives along with freezing and thawing of the serum samples tend to give unreliable readings.

It has been shown by Moore et al.\textsuperscript{2} that the test is measuring a circulating abnormal protein occurring as a result of the virus of equine infectious anemia. Russell et al.\textsuperscript{5} has shown that this abnormal protein fluctuates as the clinical signs fluctuate. One of the inherent disadvantages of the test is the fluctuation of this antigen but from the diagnostic point of view this phenomenon helps in evaluation of the stage of infection. In the acute, subacute and chronic phase horses this fluctuation of protein may be at regular intervals but the inapparent carrier horse may go 60-90 days negative to the test. As pointed out, no amount of increasing readability and sensitivity can overcome this difficulty.

Correlation of the precipitin test with horse inoculation tests were 100 percent as long as we were dealing with the highly virulent virus\textsuperscript{5} and using horses as the test animal. Some 35 horses were tested under these circumstances with complete agreement. As we began to uncover cases of a milder type virus and to use ponies as test animals, difficulties have arisen. These difficulties will be discussed later under horse inoculation tests.

CHARACTERISTICS OF THE ABNORMAL PROTEIN

The abnormal protein is produced as a result of infection with the virus and has not been found in normal horses. The protein is produced in infected leucocyte cell cultures and is serologically the same as that occurring in the circulation of the horse. In correlation we could assume that it is produced by the leucocyte of the infected horse.

Column chromatographic analysis of the cell culture fluids show three distinct peaks when readings are made on the DU Spectrophotometer at a reading of 280 wavelength. The first fraction contains a large amount of nucleic acids as shown by the 260/280 wavelength ratio. The second fraction is principally protein with little or no nucleic acids. The third fraction contains the indicator of the medium, amino acids, vitamins and other constituents of mediums. From these results it appears that we are separating virus from abnormal protein and these from the culture medium constituents. Fraction two is highly antigenic when inoculated into rabbits but fraction three is not antigenic.

Fraction two hemagglutinated chicken red blood cells in the manner described by Dreguss & Lombard.\textsuperscript{1} Antifraction two rabbit serum will specifically prevent hemagglutination of chicken red cells as well as block the hemagglutination by infected horse serum. From these results it
appears that the precipitin test and Dreguss & Lombard's hemagglutination test are measuring the same phenomenon.

The hemagglutinin test was abandoned by Dreguss because of two reasons. The first was that it would fluctuate positive and negative on a known positive horse as does the precipitin test. The second reason was that it was not specifically inhibited by another infected horse serum. They thought they were measuring viral hemagglutinin, but in light of new findings we can assume that they were adding more antigen to the system instead of antibody. If they had used serum of an inapparent carrier horse results would have been different.

The abnormal protein fluctuates in direct correlation with the temperature of a subacute or chronic horse as shown by Russell et al. It was also interesting to note that the inapparent carrier horse has a rather dramatic gamma globulin response as the abnormal protein appears. From this finding it would appear that the abnormal protein, and a lack of immunologic response in the acute, subacute and chronic horse, is the apparent cause of clinical signs becoming apparent.

The abnormal protein has a high affinity for the red blood cells. Moore et al. showed that it was on approximately 20 percent of the red cells of an infected horse by fluorescent antibody. Also the hemagglutination of chicken red blood cells by infected horse serum and fraction two of leukocyte cell cultures adds to this observation. The specific blocking of hemagglutination by hyperimmune antisera indicates that the material is highly specific in its action. This mechanism has been proposed by Moore et al. as that which produces anemia in the infected horse. The attached protein probably renders the red cell nonfunctional and it is metabolized out of the circulation as any other nonfunctioning erythrocyte.

The abnormal protein migrates on paper electrophoresis in the vicinity of the gamma fraction. Particle size is much smaller than the gamma though, approaching the albumin fraction in size. From the size of the particle, it should appear in the urine and it has been demonstrated there. This is one mechanism by which the horse may remove the abnormal protein fraction.

All of these results indicate that a circulating abnormal protein which is probably produced by the leukocyte occurs in an infected horse. The abnormal protein attaches to the blood cell and also is eliminated in the urine. It is probable that the attached abnormal protein causes the anemia and maybe other clinical signs since it appears before a temperature rise appears. The precipitin test and Dreguss & Lombard's hemagglutination test measure this abnormal protein.

HORSE INOCULATION TESTS

The last thing I would like to discuss is horse inoculation studies involved in evaluating this test. It has been assumed that this is an absolute method for diagnosis. I am discussing this test because of some peculiar reactions we have had. It is important to note these reactions because any serologic test developed must be compared to and withstand the rigors of
horse inoculation. The following are only examples of some problems. Similar incidents have occurred all too frequently.

The first discrepancy encountered in horse inoculation tests is in horses inoculated with a low virulent virus. In this case, the receptor horse cycles with a body temperature rise, but rarely if ever goes over 102°F (the temperature arbitrarily used as normal). The horse does show signs of edema as the body temperature rises and there is a progressive loss of body weight over a long period of time even under ideal conditions of providing shelter and feed. These horses finally die in a severely emaciated state some three to five months after challenge. Under standard horse test, arbitrarily taking 40 days as an incubation period, they would be regarded as negative. When this horse dies or is killed, he shows microscopic lesions compatible with equine infectious anemia.

The second discrepancy encountered in horse tests is the use of the pony as a test animal. Some ponies are apparently more resistant to the virus than others. We have seen some ponies which are negative, by all means possible to establish these as negative, which are completely refractory to challenge. Others, when inoculated, have a prolonged incubation period. For example, two ponies were inoculated with blood collected from a donor horse at one time. One of these ponies showed a body temperature rise at 14 days but the second pony did not respond with a body temperature rise until the 48th day. If the second pony had been the only pony inoculated and had been challenged on the 40th day, this would have been recorded as a mistake by the precipitin test. In our opinion the pony is a poor receptor animal.

The third discrepancy that may be encountered is that a horse showing a typical clinical response may subsequently respond with clinical signs to challenge from another donor. This phenomenon is adequately reported in the literature. The assumption has been that there is only one serologic strain of virus but these results would make one suspicious of the contrary.

**SUMMARY**

The precipitin test is measuring a circulating antigen which is probably the same as the hemagglutinin described previously. Withstanding certain reading difficulties it is a useable test for diagnostic purposes. Due to certain inherent disadvantage, mainly fluctuation of the circulating antigen, the test will never become useable as an eradication test. The test has advanced knowledge on the disease in the horse and with this knowledge it will be possible to develop a highly specific test. This will probably be a fluorescent tagged antibody test for the circulating virus. If this does not work then a mixture of tests (such as precipitin, clumping test for antibody etc.) may be needed to detect the inapparent carrier, a difficult but important animal to detect. The precipitin test has demonstrated that the incidence of inapparent carriers is much higher than anticipated.
Horse inoculation tests are highly accurate when the virulent virus is used as an inoculum and horses are the host receptor. The horse inoculation test leaves something to be desired though with viruses of low virulence and when ponies are the host receptor.

REFERENCES

Research interest in Infectious Anemia of horses (EIA) was renewed recently by confirmed outbreaks of the disease in race horses in numerous states. This culminated in a meeting with the House Appropriations Sub-Committee for Agriculture. A sum of $200,000 was appropriated for research on this disease, one-half of which was given to the National Animal Disease Laboratory at Ames, Iowa; the other half was divided among Texas A and M University, Louisiana State University, and Washington State University. Research in this area is also being conducted in many other institutions of the world. Every scientific resource must be utilized if the problems of diagnosis, control, and eradication are to be answered.

To assist in this research effort, the following bibliography was prepared to supplement the comprehensive review of the literature published by M. F. Degress and L. S. Lombard in 1954.

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The assistance of Susan Wright and Howard Woodward in the preparation of this bibliography is gratefully acknowledged.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

J.L. O'Harra, Reno, Nevada, Chairman; O. R. Adams, Fort Collins, Colorado; C. B. Dearborn, Concord, New Hampshire; L. P. Doherty, Lexington, Kentucky; M. Fowler, Davis, California; J. B. Healy, Jacksonville, Florida; R. J. Henshaw, Frankfort, Kentucky; W. O. Kester, Golden, Colorado; H. C. King, Olympia, Washington; R. O. Knowles, Hyattsville, Maryland; F. D. Maurer, College Station, Texas; W. R. McGee, Lexington, Kentucky; W. L. Sippel, Kissimmee, Florida

This Committee, in its second year of functioning, reaffirms that Piroplasmosis, infectious anemia, respiratory diseases and continued protection from African Horse Sickness are most significant to the horse population of the United States. The Committee also reaffirms that diseases with these characteristics can be controlled only on a national level and that continued and increased efforts should be oriented under the direction of developing effective tools for a national eradication and protection program. Progress has been made in all areas in the past year with significant results.

Current disease status reports and recommendations of this Committee are as follows.

CURRENT STATUS OF EQUINE PIROPLASMOSIS

Equine Piroplasmosis (EP) caused by Babesia caballi was first diagnosed in the United States in Florida in 1961. Outside of Florida, EP has been diagnosed in horses on only one premise in Southern Georgia. The Georgia herd was eradicated. A total of 150 confirmed (organism demonstrated in erythrocyte) cases have been diagnosed in Florida with five cases in 1965 and the last diagnosed case on June 25, 1965. In Florida 126 cases were diagnosed in 1962.

All living reactor horses have been treated with one of the three experimental drugs (phenamidine, berenil, diamprom) and have been released to former owners or their agent. At present these drugs are available for experimental use only. It is demonstrated that when these drugs are used individually at the rate of phenamidine 4.0 mg. per pound, berenil 5.0 mg. per kg., diamprom 4.0 mg. per pound of body weight on two successive days they can eliminate the carrier state associated with B. caballi. The drugs are administered intramuscularly at several sites. To date, only one premise remains under quarantine for EP in Florida. Dermacentor nitens (Tropical horse tick) is at this time the only proven vector of EP in the United States.

A sharp decline in the number of EP cases was observed upon initiation of the vector control program in 1962. In south Florida, spraying with 0.5 percent toxaphene and application of one percent lindane in oil to the
ears and false nostrils every 21 days has been found to effectively control tropical horse tick infestations, thereby preventing transmission of *Babesia caballi* to susceptible horses. In 1965 Piroplasmosis caused by *Babesia equi* was recognized in South Florida.

Development of reliable antigens in sufficient quantity would enable a survey to be conducted for EP carriers on horses outside of Southern Florida. Results of the survey would determine the need for extending the vector control program to other areas.

**Recommendations of this Committee**

1. That support be given to an all-out effort by the United States Department of Agriculture to produce reliable antigens in sufficient quantity for survey purposes.
2. Recognition of an official serological test.
3. Continued support be provided to conduct a survey for EP in the United States, particularly where there are proven vectors (*D. nitens)*.
4. Continue to evaluate chemotherapeutic agents for the prevention, control and treatment of EP caused by either *Babesia caballi* or *Nutallia equi*.
5. That the current vector survey (cooperative State-Federal) of equidae in the United States be continued, with increased effort in the Gulf Coast area.

**Recommended Procedures for the Unconditional Release from Quarantine of Equines Known to Be Infected with Equine Piroplasmosis**

1. Infected horses will not be eligible for consideration for release from quarantine for at least 45 days after treatment with drugs which might conceivably influence serological reaction or presence of piroplasma organisms.
2. In order to be eligible for "consideration from release from quarantine," a previously infected horse must be:
   a. Apparently healthy on physical examination and clinical observation.
   b. Negative to blood smear examination.
   c. Free from suspicion of disease (EP) by an acceptable test.
3. Animals eligible for "consideration for release from quarantine" under No. 1 and 2 above, will be further tested by subinoculation of 500 ml. of whole blood into a splenectomized recipient horse or, in lieu thereof, two mature recipient horses (seven years or older). All recipient horses must be negative to equine piroplasmosis prior to inoculation (as under No. 2 above).
4. During the test period, the recipient test horses shall be subjected to regular clinical examination and peripheral blood smear examination. If peripheral blood smear examination reveals the presence of piroplasma organisms, the donor animal will be considered infected and will not be released from quarantine except to immediately go to slaughter. If the recipient horse
remains blood-smear negative during the 45 day test period, but shows clinical signs of equine piroplasmosis or other diagnostic evidence, the donor will remain under quarantine, pending completion of additional animal inoculations to prove otherwise.

5. If the recipient test horse remains negative, it shall be challenged by inoculation with blood from a known equine piroplasmosis infected animal. If a challenge with known infected blood fails to produce equine piroplasmosis in the recipient animal, the donor will remain under quarantine, pending completion of additional animal inoculations to prove otherwise.

Reactors may be released to go directly to slaughter at any time by obtaining proper permit.

**Recommended Procedures for Releasing Equine Piroplasmosis Quarantine Premises**

1. Premises on which the infected animal and all other horses have been removed for a minimum of six months:
   a. Tick-free test horse or horses placed on the premises.
   b. Premises may be released from quarantine if test horses remain free of ticks (*D. nitens*) for 30 days and after Step C is completed.
   c. Premises sprayed with one percent toxaphene (it may be impossible to spray a complete premise but it is advisable to at least spray the stables and as much of the fence lines as possible.)

2. Premises on which the reactor has been removed for six months but on which other horses are present:
   a. Horses present on the premises have been sprayed every 21 days and no ticks (*D. nitens*) have been found the last six months.
   b. Tick-free test horses placed on the premises*
   c. If test horses remain tick-free (*D. nitens*) for 30 days, premises may be released after Step D is completed.
   d. Sprayed as under Step 1-C above.

3. Premises on which reactors are present:
   a. All reactors must be proven free of EP (by previously approved methods) at least six months before starting Step C.
   b. Horses present on premises have been sprayed every 21 days and no ticks (*D. nitens*) have been found for the past six months.
   *c. Tick-free test horses placed on the premises.*
   d. If test horses remain free of *D. nitens* for 30 days and after spraying of premises with one percent toxaphene, the premises may be released from quarantine.

*Resident horses may be used as test animals when they are found free of ticks on an inspection made at least 21 days following the last application of a tickicide.

N.B. The above procedures have been used successfully in Florida since the summer of 1963.
CURRENT STATUS OF EQUINE INFECTIOUS ANEMIA

Considerable effort has been expended this past year in the investigation and control of Equine Infectious Anemia. Unfortunately a practical test for EIA that would be acceptable for a regulatory program has not, as yet, been developed.

The Committee recognizes the value of the Prospectus on Equine Infectious Anemia with Guidelines as applied to outbreaks of the disease at racetracks in 1966. This document appears elsewhere in these Proceedings. See pages 245-254 of this Proceedings.

AFRICAN HORSESICKNESS

The report of the Committee on Foreign Animal Diseases in this Proceedings, documents recent outbreaks of African Horsesickness in Africa. This intensifies the need to take steps to prevent the introduction of this devastating scourge of equidae into the United States.

EQUINE RESPIRATORY DISEASES

The Committee commends the equine industry's support of research in the area of respiratory diseases.
COMPARATIVE PROTECTION TESTS OF DIFFERENT COMMERCIAL ANTI-HOG CHOLERA SERUMS AGAINST REGULAR AND VARIANT HOG CHOLERA VIRUSES

J. P. Torrey, B.S., D.V.M., M.S.; M. R. Zinober, D.V.M.*

Reports of the results of controlled experiments relative to the comparative potency and dosage of hog cholera (HC) antiserum and virus have been limited. Cole et al.² state that 10 ml each of two commercial HC antiserums was the minimum dose of serum that afforded complete protection against two ml of regular virus. This same amount of serum protected against 100 ml of virus, indicating that the in vivo reaction which occurred between serum and virus in simultaneously treated hogs was not a quantitative one. Ten ml of these same serums did not protect pigs against two ml of variant virus.

The dosage of serum has been determined largely by the results obtained in the field. Producers have increased the recommended dose from time to time since serum was first used for immunizing pigs. Some practicing veterinarians purchased more than one serial lot of serum of a producer and mixed them before administration. Other veterinarians have mixed serums from different producers. While these procedures gave better results, there was no obvious reason for the improved protection which resulted.

Dale et al.³ proved that the outbreaks of HC after vaccination in 1949-50 were due to the use of particular serial lots of serum and virus which in turn were associated with what was identified as a variant form of HC virus.

Present Federal regulations¹ for the production of HC antiserum require a potency test in which 15 ml of serum is used with two ml of virus in pigs weighing less than 90 lbs. Pigs weighing over 90 lbs. are given 20 ml of serum. While this test provides for the release of serums that are not below the prescribed standard, it gives no indication of the comparative potency of individual serum lots.

The objective of this investigation was to compare the protective properties of eleven commercial HC antiserums against regular and variant HC viruses.

*From the National Animal Disease Laboratory, Animal Disease and Parasite Research Division, U. S. Department of Agriculture, Agriculture Research Service, Ames, Iowa.
MATERIALS AND METHODS

Serums.—A supply of one serial lot of HC antiserum was purchased from each of eleven commercial producers and numbered from 1-11 for identification. They were held in refrigeration at 4°C. The gamma globulin content of each serum was determined by electrophoresis.*

Viruses.—The two types of HC virus used in these experiments were: (1) regular virus, SN304, had been used at the Ames, Iowa, Hog Cholera Research Station;** the results of repeated tests had proved it to be free from variant characteristics and to have 5x10^5 infectious doses for swine/ml.

(2) Variant virus, 30, which was isolated from a herd of pigs that sickened after vaccination with crystal violet glycerol vaccine. Repeated tests of this virus proved it to be a variant type and to have 1x10^6 infectious doses for swine/ml.

Experimental animals.—One hundred twenty-eight head, comprising eight lots of Hampshire pigs weighing 20-80 pounds (average 47.6 pounds), were used in these experiments. The pigs were purchased from farms where vaccination for HC was not practiced and where previously purchased pigs were found to be susceptible to HC and free from other swine diseases. The pigs from a farm were designated as a lot and given a number. The pigs in each lot were about the same age although there was considerable variation in size.

Six pigs from two or more lots were used to test each serum with regular virus and the same number were used with variant virus. In two instances pigs were not available to test the largest doses of serums, Nos. 2 and 3, with variant virus. Two pigs, each from different lots, were used to test each size dose of serum.

A serum was tested in 10, 5, and 2.5 ml doses when injected simultaneously with two ml of regular virus and in 15, 10, and 5 ml doses when injected simultaneously with five ml of variant virus. Because of the difference in titer, a greater volume of variant virus was used. Larger volumes of serum were used to protect against variant virus than against regular virus.2 Simultaneous injections of serum and virus were made subsutaneously on opposite sides of the pig, posterior to point of the elbow. The six pigs injected with each serum sample were housed in an isolated pen. Daily observations were made and the condition of each pig was recorded by a point system, the results of which expressed the "percent protection" for the individual pig and for a group of pigs.4

RESULTS

A comparison of the test results for each serum with regular and variant virus is shown in Tables 1 and 2. The mean percent protection

*The gamma globulin determinations were made at the Armour and Co. Research Laboratory, Chicago, Illinois.

**The station was closed in 1961 and research on hog cholera is being conducted at the National Animal Disease Laboratory, Ames, Iowa.
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**"+"** sign indicates the smallest protective dose per pound weight was not reached so it is more than the amount given.

**TABLE II**

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Smallest Protecting Dose (ml)

| 2.5 | 2.5 | 5 | 5 | 5 | 2.5 | 2.5 | 2.5 | 2.5 |

Smallest Protecting Dose (ml)

Per Pound Weight

| .05 | .04 | .10 | .10 | .10 | .09 | .05 | .08 | .06 | .05 | .08 |

-"**" sign indicates that the pig had a reaction.

* The Denominator = Number of pigs treated.
The Numerator = Number of pigs that survived.
conveyed by the serums against regular HC virus ranged from 47 to 100 and against variant virus from 17 to 50. The smallest protective dose of the serums against regular HC virus ranged from 2.5 to five ml and against variant virus from 10 to more than 15 ml. There were seven serums that did not protect pigs in 15 ml doses against variant virus without a reaction, therefore, the minimum protective dose was not determined.

On the basis of dosage of serum per pound of body weight, the smallest amount of serum that protected a pig without signs of a reaction against regular HC virus ranged from .04 to .22 ml/lb. The smallest amount that protected a pig without signs of reaction to variant HC virus ranged from .17 to .50+ ml/lb. for 10 of the serums. One serum, No. 4, did not give this level of protection.

**Serum and regular virus.**—Eight of the serums gave 60 percent or more protection and three serums, Nos. 3, 9 and 10, gave less than 60 percent protection when they were injected simultaneously with regular virus. Three serums, Nos. 1, 2 and 11, gave very high percent protection with regular virus. Serum No. 1, at all three dose levels, protected all pigs from detectable reaction to regular virus inoculation, thereby giving 100 percent protection. Two serums, Nos. 2 and 11, gave 84 and 83 percent protection, respectively.

Sixteen pigs (24.2 percent) died in this experiment when regular HC virus was used. Twelve had received 2.5 ml of serum, three had received five ml of serum and one had received 10 ml of serum. One or more pigs died in nine of the serum tests. Survival with reaction was seen in eight serum tests. No pig died in tests of serum Nos. 1 and 6, one pig died in tests of serum Nos. 2, 4, and 11, two pigs died in tests of serum Nos. 5, 7, 8, 9, and 10, and three pigs died in the test of serum No. 3 (Table 2).

Four hundredths ml/lb. body weight was the smallest amount of serum that protected a pig without detectable reaction, serum No. 2. This, however, was not the serum that gave the best protection in all the pigs; serum Nos. 1, 7, and 11 protected pigs without a reaction in .05, .05 and .08 ml doses per pound weight, respectively. However, serum Nos. 1 and 11 gave notably better mean protection of the other pigs in the test than did serum No. 7. Serum Nos. 6, 8, 9, and 10 prevented pigs from dying in less than .1 ml/lb. amounts but the surviving pigs had reactions.

**Serums and variant virus.**—The percent protection was less than 60 for each of the serums when used with variant virus (Table 2). Serum Nos. 3 and 6 gave 50 percent protection and serum Nos. 1 and 2 gave 47 and 45 percent protection respectively. Serum No. 4 was the least effective in protecting pigs against variant virus. Five of the pigs receiving serum No. 4 died and the sixth pig had a severe reaction. Only one serum, No. 3, had a higher percent protection (50 percent) with variant virus than with regular virus (47 percent).

Thirty-four pigs (54.8 percent) died when variant virus was used
with these serums. Twenty-one of the dead pigs had received five ml of serum, 10 had received 10 ml of serum and 3 had received 15 ml of serum. One serum, No. 3, protected both pigs from death or visible illness when given at a 10 ml dose. One other serum, No. 11, protected both pigs from death or visible illness when given at a 15 ml dose. The other nine serums did not protect the pigs from visible illness when given at 15 ml doses. The smallest amount of serum per pound weight that protected the pigs against variant virus without a reaction was .17 ml/lb., serum No. 2; however, the other pig on this same volume of serum received .20 ml/lb. and died. Other serums prevented death of pigs when .20 to .50+ ml per pound weight was given but the surviving pigs had severe reactions.

DISCUSSION

The largest dosage of the eleven serums used in this study with regular virus prevented one or both pigs from dying in each test. Ten ml doses of seven serums protected both pigs against regular virus without any reaction but only two serums protected both pigs against variant virus in the highest dosages used. In the tests of 10 serums, two to four times more serum per pound weight was required to protect pigs against variant virus than against regular virus. These results substantiate the report of Dale et al.³

The recommended dose of serum to be used with modified vaccines under field conditions varies with the producer. The results of this experiment indicate that unless a high titer serum is used, it is not safe to give less than a 10 ml dose of serum. If there is a possibility of variant virus being present, this dosage should be doubled.

The veterinarian who mixed serials or serums from different producers was taking less chance of using a serum of low protective properties. If serum of standard titer had been available, mixing would not have improved the protective properties.

The results of this experiment also point out the need for better standard methods in the production and testing of HC antiserum. If serums of more uniform high potency were produced, the recommended dose could then be reduced. The smallest amount of serum per pound body weight that protects the pig against HC virus is not always indicative of the protection that serum will have for other pigs. Two serums used in this experiment protected pigs in the same amounts per pound body weight; one serum protected all of the six pigs on the test and the other serum protected three of the six pigs on the test.

The mean percent protection when obtained from a group of pigs given graded doses of serum seems to give a more realistic figure for the evaluation of protective properties of HC antiserums.

Dale et al.³ after extensive tests found a serum which would protect pigs against regular virus in 15 ml doses but would not protect against variant virus and proposed such serums for detecting viruses
with variant characteristics. Serum No. 4 in this experiment was a good example of a serum which may be used for detecting variant viruses.

SUMMARY

The protective properties of eleven commercial anti-hog cholera (HC) serums were tested in graded doses with regular and variant HC viruses. The percent protection conveyed by the serums against regular HC virus ranged from 47 to 100 and against variant virus from 17 to 50.

The smallest amount of serum per pound body weight that protected a pig without signs of a reaction to regular HC virus ranged from .04 to .22 ml. and to variant HC virus from .17 to .50+ ml.

REFERENCES

HETEROTYPIC HOG CHOLERA PROTECTION IN SWINE:
AN ANALYSIS OF THE RESPONSE

F. J. Volene, B. E. Sheffy and J. A. Baker

Heterotypic protection has been defined as the resistance that can be induced against a specific viral disease by inoculation of a different virus that does not cross-neutralize with the infective virus but does share certain essential characteristics of the group of viruses to which both belong. This protection seemed to be based upon ability of the heterotypic virus to adapt cells into a condition of secondary response, which then enables these cells to accelerate their production of antibodies after exposure to the viral disease. Such heterotypic response was found originally in protection of pigs against hog cholera (HC) by bovine virus diarrhea (BVD) virus of dogs against canine distemper by measles virus from human beings and of dogs against infectious canine hepatitis (ICH) virus by adenovirus from human beings.

For characterization of antibody formation, two phases have been postulated. Following a single inoculation, cells became adapted for antibody production in the first phase, while antibody formation was considered the second phase. When the same antigen was inoculated again, antibody was accelerated and this phenomenon has been called secondary response. Relationship of antibody type in a primary respondent and in secondary response have been studied. As illustrated by response of pigs, early antibody was found to be of 19 S type while antibody formed later was mainly 7 S. After sequential inoculation of the same antigen, further antibody production was found mainly of 7 S type.

When antigen was homologous and nonreplicating, phases of antibody responses were related to quality of antigen and time sequences following multiple inoculations. When antigen replicated as with live viruses, the two phases blended. Because live BVD virus induced detectable antibody to itself but not HC virus yet induced accelerated antibody when HC virus was given sequentially, the type of antibody following this heterotypic secondary response was analyzed.

MATERIALS AND METHODS

Virus.—A non-cytopathogenic bovine virus diarrhea (BVD) virus, strain New York-1, was used for initial inoculation into pigs. From an infected calf spleen, virus that had been transferred one or two times was inoculated into bovine kidney cell cultures maintained with Earles' balanced salt solution, 0.5 percent lactalbumin, four percent bovine serum and antibiotics. Cultures were incubated at 37°C and six days
after inoculation fluids were harvested, pooled and stored at -70°C until used. Prior to inoculation into pigs, pooled virus was titrated in tissue-cultured bovine kidney cells by the interference method, in which 100 TCID₅₀ of National Animal Disease Laboratory cytopathogenic BVD strain was used as challenge virus. Challenge of protection made use of strain A virulent HC virus which was inoculated as a 10 percent spleen emulsion from an infected pig.

**Animal Inoculation.**—Pigs were obtained from the specific pathogen free (SPF) herd maintained at the Institute and groups of five were placed in isolation units. Each pig in a group was inoculated either intramuscularly or intravenously with 10 ml. of BVD virus that titrated log 5.8 per ml. Beginning at the time of inoculation with BVD and daily thereafter, for five days, a group was selected and each pig was given intramuscularly one ml. of HC virus suspension. Pigs were observed for signs of illness and temperatures were taken daily. At the time HC was inoculated and at four, seven, 10 and 14 days serum samples were taken and stored until tested.

**Antibody Studies.**—In order to determine whether antibody was 19 S or 7 S, the procedure followed was that outlined by Deutsch and Morton. Each serum sample was separated into two portions. One portion was mixed with an equal volume of 0.2 M. 2-mercaptoethanol (2-ME) while the other was mixed with an equal volume of tissue culture medium. Mixtures were incubated for 30 minutes at 37°C and then placed at room temperature overnight in order to allow for evaporation of the 2-ME. All samples were adjusted to a final dilution of 1:5 and then tested for neutralizing capacity against BVD virus and HC virus.

Five-fold dilutions of each serum portion were prepared with Earles' maintenance medium. Then part of each dilution was mixed with an equal volume of BVD virus suspension that had been titrated to contain 100 tissue culture infectious doses (TCID₅₀) per 0.1 ml. and the other part with HC virus that had been titrated to contain 100 TCID₅₀ per 0.1 ml. Serum-virus mixtures were shaken and placed at 37°C for 30 minutes. Then each of three tubes of bovine kidney cell cultures was inoculated with 0.2 ml. of a mixture and placed at 37°C. Tubes were examined daily for seven days and serum titers were calculated according to the Spearman-Karber method.

**RESULTS**

Swine failed to survive virulent HC virus when BVD virus had been inoculated at the same time and one or two days previously; two of five pigs survived when BVD had been inoculated three days previously; three of five pigs when BVD virus had been given four days prior to HC virus; after five days all five pigs survived. A 50 percent protection period was calculated to be 3.5 days (Table I).

When the interval of time between sequential inoculation of BVD
virus and HC virus was five days, antibody against BVD virus and against HC virus were detected ten days after HC virus had been given. When the interval of time was five weeks, HC antibody was detected seven days after inoculation of HC virus and BVD antibody showed a marked increase (Figure 1). Accelerated antibody treated with 2-ME to inactivate 19 S showed titers against BVD virus (Figure 2) and against HC virus (Figure 3) that were similar to antibody titers in untreated serum, except titer of HC antibody seven days afterwards showed more antibody in untreated serum while 10 days afterwards they were similar.

**DISCUSSION**

The time interval for induction of heterotypic protection against hog cholera by BVD virus was found to be similar to that reported when

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**TABLE I**

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<tr>
<td>4</td>
<td>3/5</td>
</tr>
<tr>
<td>5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

---

Figure 1. Detectable antibody in relation to time interval between sequential inoculation of BVD virus followed by hog cholera virus.
Figure 2. Relationship of 7 S antibody (solid bar) to total BVD antibody (plain bar) after sequential inoculation of BVD virus followed by hog cholera virus.

Figure 3. Relationship of 7 S antibody (solid bar) to total HC antibody (plain bar) after sequential inoculation of BVD virus followed by hog cholera virus.
attenuated HC virus preceded virulent HC virus, that is, 3.5 days were required for 50 percent protection. No antibody to BVD was found at this time and full capacity to accelerate antibody was not reached until later. When the time interval of five days between sequential inoculation of BVD followed by HC virus was compared with the five week interval, the appearance of detectable antibody was shortened from ten to seven days at the longer time interval. After a single inoculation of live attenuated HC virus, 14 days were required for formation of detectable HC antibody. Even at the shorter time interval between sequential inoculation of BVD virus followed by HC virus, measurable antibody production was accelerated.

A single inoculation of inactivated HC virus provided protection against hog cholera by adaptation of cells for secondary response. In this respect, inactivated HC virus behaved like heterotypic BVD virus which accelerated antibody of the 7 S type following a second inoculation of HC virus. Also, accelerated antibody produced by sequential inoculation of attenuated live HC virus followed by virulent HC virus was shown to be of 7 S type (Figure 4). Correlation of time intervals for appearance of 7 S antibody after a single inoculation indicated that 7 S antibody would be expected after the sequential inoculations. Antibody produced by homologous and heterologous inoculation procedures between measles and distemper indicated that the capsids of these viruses have similar antigens, but that there was no common identical antigen. Adsorption of antibody onto, and elution from, preparations of purified measles and distemper virus indicated that at least part of the antibody produced during the heterologous secondary response comprised antibody that reacted with both viruses. Invocation of 7 S HC antibody by prior inoculation of BVD virus indicated that BVD virus and HC virus, like distemper and measles, may share similar antigenic components.

![Figure 4. Relationship of 7 S antibody (solid bar) to total HC antibody (plain bar) after two sequential inoculation of hog cholera virus.](image-url)
For 50 percent protection of pigs against HC virus by BVD virus, prior inoculation of 3.5 days was required. When time intervals between sequential inoculations of BVD followed by HC virus were compared, an interval of five weeks accelerated detectable HC antibody within seven days whereas detectable HC antibody was not found until ten days afterward when the time interval was five days. Following a single inoculation of HC virus, antibody appearance required 14 days. In the heterotypic antibody response, BVD antibodies were accelerated and increased following HC virus inoculation and were of the 7S type. Also, HC antibody found 10 days afterwards was of 7S type.

ACKNOWLEDGMENTS

The excellent technical assistance of Mrs. Lillian Sherman and Mrs. Marion I. Bowman is gratefully acknowledged.

REFERENCES


SAFETY TESTING HOG CHOLERA LIVE VIRUS MODIFIED VACCINES

C. E. Phillips, D.V.M.
Ames, Iowa

INTRODUCTION

The hog cholera eradication program has stimulated a new interest in the safety of live virus immunizing agents. Involved are field reports of hog cholera breaks following immunization, the spread of vaccine virus to contact shoats and pregnant sows, and the use of serum simultaneously with live virus modified (LVM) vaccines. These questions are pertinent and important to the eradication program. This study was started in an attempt to resolve some of these questions.

In previous work (unpublished) eight LVM vaccines, seven tissue culture and one rabbit, were titrated and studied for spreading characteristics. Tests without anti-hog cholera serum showed the vaccine spread to be zero percent to 48 percent. The pigs used in these tests were considered to be hog cholera susceptible test pigs although not checked serologically. The test indicated that the vaccine virus spread is proportional to the virulence of the vaccine virus.

Test #1 (Table I) demonstrated that one vaccine spread equally with or without serum. This indicated that if the vaccine produced a hog

| TABLE I |
| Vaccine Spread and Titration of a Tissue Culture Origin Vaccine |

<table>
<thead>
<tr>
<th>DESIGN</th>
<th>UNDILUTED</th>
<th>10^-1</th>
<th>10^-2</th>
<th>10^-3</th>
<th>10^-4</th>
<th>CONTACT PIGS IMMUNIZED BY EXPOSURE TO VACCINATES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WITH 10 ML. SERUM</td>
<td>VACCINATES</td>
<td>5/5 *</td>
<td>5/5</td>
<td>5/5</td>
<td>4/5</td>
<td>4/5</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
<td>5/5</td>
<td>3/5</td>
<td>5/5</td>
<td>1/5</td>
<td>0/5</td>
</tr>
<tr>
<td>WITHOUT 10 ML. SERUM</td>
<td>VACCINATES</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>4/5</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
<td>5/5</td>
<td>5/5</td>
<td>1/5</td>
<td>4/5</td>
<td>4/5</td>
</tr>
</tbody>
</table>

* SURVIVORS, POST-CHALLENGE/TOTAL CHALLENGED

EIGHT VACCINES WERE TESTED (7 TISSUE CULTURE ORIGIN AND 1 RABBIT ORIGIN) USING THE SAME TEST DESIGN (WITHOUT SERUM). THE PIGS WERE HOG CHOLERA SUSCEPTIBLE TEST PIGS NOT SEROLOGICALLY CHECKED. THESE TESTS SHOWED THE VACCINE SPREAD TO BE FROM 0% TO 48%. THE TESTS INDICATED THAT THE VIRUS SPREAD IS PROPORTIONAL TO THE VIRULENCE OF THE VACCINE.

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cholera syndrome in vaccinates without serum, the product could be
dangerous to all contact unvaccinated pigs even when used with serum.
Therefore, it was decided to evaluate vaccines at the recommended im-
umunizing dose and without the simultaneous use of serum. It was con-
cluded that hog cholera LVM vaccine virus spreads equally with or without
the simultaneous use of anti-hog cholera serum.

Pigs used on these initial LVM vaccine titration and spread experi-
ments were unvaccinated, 50 pound shoats, female and male (castrated)
selected from a farm which also supplied a biological producer with test
pigs. Five pigs from the herd were challenged with two ml. of virulent
hog cholera virus (Lot AIQ #1) to determine susceptibility.

Isolation units providing complete isolation were used to accommo-
date 10 pigs for each dilution of vaccine virus. One serial was evaluated
at a time using the recommended vaccine dose, two ml., in dilutions of $10^0$
to $10^4$. Five pigs were vaccinated and five contact controls were used for
each dilution. The pigs were observed daily for inappetence and tempera-
tures were taken and recorded. The pigs in tests one to eight were chal-
lenged 21 days post vaccination; the pigs in test nine (use of serum versus
no serum) were challenged on the 28th day. The criterion for immunity
was freedom from the symptoms of hog cholera, i.e., elevated tempera-
ture, inappetence, death and the presence of hog cholera lesions upon
necropsy.

Fifty pigs randomly selected from the last group were tested by the
serum neutralization-fluorescent antibody (SN-FA) test. The test showed
that 54 percent of these pigs had low level antibodies to hog cholera virus.
Subsequent work has shown that pigs from selected farms can be negative,
mixed or all have a positive titer.

MATERIALS AND METHODS

Pigs selected for the vaccine safety tests were determined to be
susceptible to hog cholera by the SN-FA test. (The SN-FA test method
is being prepared for publication.)

All pigs used in the safety test were shown to be negative for hog
cholera antibody at the lowest demonstrable dilution (one to two).

The test design that was accepted as fulfilling practical requirements
consisted of:

1. Susceptible pigs, 40-60 pounds, serologically negative for hog
cholera antibodies.
2. Isolation units for each test.
3. Vaccination of six pigs with the recommended dose (two ml.).
4. Six unvaccinated contact controls, serologically negative and
randomly selected.
5. These pigs were observed for 22 ± two days. Temperatures were
recorded daily and observations for hog cholera symptoms were
made. Deaths were recorded, necropsies performed, and tissues
collected for FA determination of hog cholera virus content.
TABLE II

Hog Cholera Vaccine Safety Test
Serologically Negative Pigs

<table>
<thead>
<tr>
<th>GROUP I</th>
<th>VACCINE A</th>
<th>VACCINE B</th>
<th>VACCINE C</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ TYPE VACCINE ↓</td>
<td>↓ TYPE PIGS USED* ↓</td>
<td>↓ NUMBER ↓</td>
<td>↓ SICK ↓</td>
</tr>
<tr>
<td>PORCINE ORIGIN **</td>
<td>FARM PIGS SPF - 2ND GENERATION</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>TISSUE CULTURE ORIGIN ***</td>
<td>FARM PIGS SPF - 2ND GENERATION</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>TISSUE CULTURE ORIGIN</td>
<td>FARM PIGS SPF - 2ND GENERATION</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>TISSUE CULTURE ORIGIN</td>
<td>FARM PIGS SPF - 2ND GENERATION</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>TISSUE CULTURE ORIGIN</td>
<td>FARM PIGS SPF - 2ND GENERATION</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* KILLED 21 DAYS, POST-VACCINATION (TEMPERATURE 106.4 F)
** SALES DISCONTINUED
*** PRODUCTION DISCONTINUED

serological tests were conducted when time permitted. At 22 ± two days, the living vaccinated pigs were necropsied. Following removal of the vaccinated pigs, three additional susceptible pigs were placed in contact with the original contact pigs. These were kept in contact for 22 days. All remaining pigs were autopsied on the 45th day, laboratory test work completed and the vaccine evaluated.

The difference in the ability of vaccine virus to spread and produce a hog cholera syndrome and/or death confirmed by characteristic lesions, virus isolation, and/or serology, is well documented in the tables and graphs presented. Three vaccines, one porcine origin and two tissue culture origin (Table II), showed that the porcine origin vaccine used without serum will produce hog cholera. It can also be postulated from the symptoms produced in the contact pigs, and our knowledge of spread, that even with the simultaneous use of anti-hog cholera serum, it would pose a potential danger to all contact unvaccinated pigs. Vaccine B (Table II) tissue culture origin showed persistent virus, hog cholera syndrome and lesions with a temperature curve peaking at 106.4 F. in one contact pig. It is presumed that the virus in this vaccine presents a potential danger in a herd.

Vaccine C (Table II) tissue culture origin appears to represent a safe vaccine and to warrant further evaluation.

The average temperature curve of five pigs (Graph I) demonstrates the reaction caused by the vaccine viruses and their ability to produce a thermal response in hog cholera susceptible pigs. The thermal response of one pig, Vaccine B (Table II) is better illustrated by the peak of the temperature curve as related to all other indications of a hog cholera reaction.

Vaccine D (Table III) is a repeat test of Vaccine B (Table I). This
### Table III

**Hog Cholera Vaccine Safety Test**

**Serologically Negative Pigs**

<table>
<thead>
<tr>
<th>Type Vaccine</th>
<th>Type Pigs Used</th>
<th>Number</th>
<th>Sick</th>
<th>Average Temperature (F.)</th>
<th>Dead</th>
<th>Hog Cholera Lesions</th>
<th>Virus Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VACCINE D</td>
<td>VACCINATES</td>
<td>6</td>
<td>0</td>
<td>103.2</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td></td>
<td>TISSUE CONTACTS</td>
<td>6</td>
<td>2</td>
<td>103.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LATE CONTACTS</td>
<td>3</td>
<td>0</td>
<td>103.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VACCINE E</td>
<td>VACCINATES</td>
<td>6</td>
<td>3</td>
<td>103.9</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>TISSUE CONTACTS</td>
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<td>103.9</td>
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<td>2</td>
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<tr>
<td></td>
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<td>3</td>
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<td>3</td>
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<tr>
<td>VACCINE F</td>
<td>VACCINATES</td>
<td>6</td>
<td>3</td>
<td>104.1</td>
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<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>RABBIT CONTACTS</td>
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<td>2</td>
<td>103.5</td>
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</tbody>
</table>

* 2ND GENERATION - SPF
** PRODUCTION DISCONTINUED
*** SALES DISCONTINUED

### Table IV

**Hog Cholera Vaccine Safety Test**

**Serologically Negative Pigs**

<table>
<thead>
<tr>
<th>Type Vaccine</th>
<th>Type Pigs Used</th>
<th>Number</th>
<th>Sick</th>
<th>Average Temperature (F.)</th>
<th>Dead</th>
<th>Hog Cholera Lesions</th>
<th>Virus Isolation</th>
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<tr>
<td>VACCINE G</td>
<td>VACCINATES</td>
<td>6</td>
<td>5</td>
<td>104.2</td>
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<td></td>
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<td>103.5</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>LATE CONTACTS</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>VACCINE H</td>
<td>VACCINATES</td>
<td>6</td>
<td>6</td>
<td>104.7</td>
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<tr>
<td></td>
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<td>6</td>
<td>104.0</td>
<td>3</td>
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<td>2</td>
</tr>
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<td></td>
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<td>2</td>
</tr>
<tr>
<td>VACCINE I</td>
<td>VACCINATES</td>
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<tr>
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<td>5</td>
<td>103.8</td>
<td>3</td>
<td>4</td>
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<td>3</td>
<td>104.5</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* SPF - 2ND GENERATION
** SALES DISCONTINUED

vaccine showed a persistent viremia in three out of five pigs, indicating a potentially dangerous virus. Based upon the results of two tests coupled with field problem reports, production of this vaccine has been discontinued. Vaccine E (Table III) was a suspect dangerous vaccine,
### TABLE V
Hog Cholera Vaccine Safety Test
Serologically Negative Pigs*

<table>
<thead>
<tr>
<th>TYPE VACCINE</th>
<th>PIGS USED</th>
<th>NUMBER</th>
<th>SICK</th>
<th>AVERAGE TEMPERATURE (F.)</th>
<th>DEAD</th>
<th>HOG CHOLERA LESIONS</th>
<th>VIRUS ISOLATION</th>
<th>SEROLOGY SN/FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VACCINE J</td>
<td>VACCINATES</td>
<td>6</td>
<td>1 **</td>
<td>103.6</td>
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<td>0</td>
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<td>...</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
<td>6</td>
<td>0</td>
<td>102.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>LATE CONTACTS</td>
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<td>0</td>
<td>102.4</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VACCINE K</td>
<td>VACCINATES</td>
<td>6</td>
<td>0</td>
<td>102.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
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<td>102.7</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>LATE CONTACTS</td>
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<td>0</td>
</tr>
<tr>
<td>VACCINE L</td>
<td>VACCINATES</td>
<td>6</td>
<td>0</td>
<td>103.5</td>
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<td>0</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
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<td>0</td>
<td>103.2</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>LATE CONTACTS</td>
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<td>0</td>
<td>102.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>...</td>
</tr>
</tbody>
</table>

* 2ND GENERATION - SPF
** DIED 16TH DAY - NO HOG CHOLERA LESIONS OR VIRUS ISOLATED.

### TABLE VI
Hog Cholera Vaccine Safety Test
Serologically Negative Pigs*

<table>
<thead>
<tr>
<th>TYPE VACCINE</th>
<th>PIGS USED</th>
<th>NUMBER</th>
<th>SICK</th>
<th>AVERAGE TEMPERATURE (F.)</th>
<th>DEAD</th>
<th>HOG CHOLERA LESIONS</th>
<th>VIRUS ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>VACCINE M</td>
<td>RABBIT ORIGIN</td>
<td>6</td>
<td>1 ***</td>
<td>103.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
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<td>0</td>
<td>103.1</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>VACCINE O</td>
<td>TISSUE CULTURE ORIGIN</td>
<td>6</td>
<td>0</td>
<td>103.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
<td>6</td>
<td>0</td>
<td>102.9</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>VACCINE P</td>
<td>TISSUE CULTURE ORIGIN</td>
<td>6</td>
<td>6</td>
<td>104.6</td>
<td>3</td>
<td>5</td>
<td>2/4</td>
</tr>
<tr>
<td></td>
<td>CONTACTS</td>
<td>5</td>
<td>4</td>
<td>103.6</td>
<td>1</td>
<td>1</td>
<td>...</td>
</tr>
</tbody>
</table>

* 2ND GENERATION - SPF PIGS
** TESTS NOT COMPLETED
*** VIRUS ISOLATED ON THE 16TH DAY (TEMPERATURE 106.8)

Based upon observation of the producer's tests and was removed from the market by agreement with the producing company after one official test.

Vaccine F (Table III) LVM (rabbit origin) based on the first test and field observation demonstrates a potential danger and its disposition is under review.
Tests on porcine origin LVM Vaccines G, H, I (Table IV) show that they produce hog cholera without the simultaneous use of hog cholera serum; therefore, sales of all porcine origin vaccines have been discontinued.

Based upon knowledge of attenuation, field reports and company test protocols, several hog cholera vaccines, LVM, considered to be safe were selected for evaluation. Table V and VI and Graph II show the results of these initial tests. Vaccines J, K, and L (Table V) and Vaccines M and O (Table VI) appear to be safe upon initial tests, whereas, initial test indicates that the virus Vaccine P (Table VI) is not sufficiently attenuated.

DISCUSSION

These tests have shown that a basic difference exists in the pathogenicity of viruses used in the production of hog cholera vaccines. This difference may be associated with the virus attenuation, or the method

AVERAGE TEMPERATURE OF 5 SELECTED FARM PIGS

GROUP I

![Graph I](image-url)
of vaccine production. Safe vaccines would appear to have a definite place in the eradication program and the use of safe vaccines could assure protection in the final phases of the program as well as insurance against spread in case of re-introduction of the disease.

The work has revealed that even the more attenuated vaccine virus can be recovered from the spleen, tonsils and other tissues of vaccinated pigs up to 14 days, though usually not after nine days. The identification of a virus either by FA or pig inoculation after that time would indicate a persistent virus and presents the danger of establishing a carrier state.

**SUMMARY**

Previous work showing the spreading potential of eight hog cholera live virus modified vaccines without anti-hog cholera serum, and one live virus modified hog cholera vaccine using anti-hog cholera serum simultaneously is reported. A test to determine the safety of hog cholera vaccines using serologically negative hog cholera test pigs was established.

The criteria for selecting safe vaccines is the absence of hog cholera symptoms in test pigs as demonstrated by temperature response, persistent virus, contact transmission, death and hog cholera lesions upon necropsy.

Some vaccines were shown to present a potential danger to the hog cholera eradication program and either have been or will be removed from the market.
ACKNOWLEDGMENTS

The author expresses his appreciation to Dr. Donald Randall, Mr. Charles Evans, and Mr. Steven Hanson for technical assistance and to Mr. Ralph Glazier for the graphic illustrations.

REFERENCES


SPREADING CHARACTERISTICS OF COMMERCIAL HOG CHOLERA
MODIFIED LIVE VIRUS VACCINES IN SWINE.
I. IN VIVO STUDIES*

M. R. Zinober, D.V.M. and L. O. Mott, D.V.M.

In 1962, Golding reported an outbreak of hog cholera (HC) in New South Wales, Australia, caused by a "mild" strain of virus with high morbidity and variable mortality. Clinical signs and postmortem lesions, although variable, were suggestive of HC. In the same year, Keast, Littlejohns and Helwig reported on the techniques used in laboratory confirmation of the disease. Although numerous tests were used, the ultimate diagnosis was more suggestive than definitive.

In this country, Zinober and Mott reported the results of 69 months' study of farm swine herds in Suwannee County, Florida, on the immunogenic efficacy of three types of commercial modified live hog cholera virus (HCV) vaccines (lapine origin, porcine origin and tissue culture) administered simultaneously with not less than 15 ml. of HC antiserum. Test results demonstrated that these vaccines could produce satisfactory immunity in swine without producing clinical evidence of sickness or outbreaks of HC. In successive years, however, analysis of data indicated a decline in the immunity of the vaccinated swine. Zinober and Berlin and Zinober et al. found this to be due, in part, to vaccine age or excessive expiration date periods. When these expiration date periods were shortened the immunity levels rose but never returned to the levels obtained during the first six months of the tests. These results suggested that modified live virus in vaccinated herds was spreading to newborn susceptible pigs before vaccination, possibly increasing their resistance to HC and making them less susceptible and at the same time making them less satisfactory subjects for maximal immune response. Immune tolerance as described by Carbrey may also be a factor in this connection. Mott, in unpublished work, has demonstrated that the ability to develop immunity is directly related to HCV susceptibility. This was borne out by the lower levels of immunity in the later years of the tests in Suwannee County, Florida, as reported by Zinober and Mott.

In the first years of the work in Florida, fiscal years 1957 and 1958, the percentages of pigs adequately protected were quite high, 96.4 and 94.3, respectively. In the succeeding years, the marked decline in protection which was observed, was associated with the use of older vaccines. This was corrected by the State of Florida and the percentage of pigs protected increased again but they never reached the same high levels as in the beginning of the program. A logical explanation for

this was that the nonvaccinated swine in the county had become less susceptible due to exposure following widespread use of modified live virus vaccines. After vaccination, they did not develop as solid immunity as when they were more susceptible. Consequently, when they were challenged, fewer were adequately protected resulting in a decrease in the percentage of pigs immunized.

The object of the work being reported here was to determine if commercial modified live HCV vaccines produced in the United States spread to nonvaccinated pigs and if this transmission did occur, whether or not it influenced the development of clinical signs of HC, perhaps in a mild form such as occurred in Australia\textsuperscript{1,2}. Another object of the work being reported here was to determine whether or not commercial modified live HCV vaccines induced immunity by contact infection. Torrey \textit{et al.}\textsuperscript{3} reported that these types of vaccines when serially passed through pigs transmitted immunizing virus, and, in some cases, upon continued passage the virus became fully virulent and indistinguishable from regular HCV.

\textbf{MATERIALS}

\textit{Vaccines}.—In order to secure representative samples of all commercial vaccines licensed for production and sale in the United States, the Animal Inspection and Quarantine Division (now the Veterinary Biologics Division) advised that there were 46 modified live virus vaccines being produced by 22 manufacturers when preparations were being made to begin these experiments. Upon attempting to purchase samples of these vaccines, however, it was learned that production of 13 of them (five lapine origin, seven porcine origin and one tissue culture) had been discontinued. All of the remaining 33 vaccines were available and were used in this work. These vaccines were designated by the Animal Inspection and Quarantine Division as being produced under different granted patents, patents pending, royalty sub-licensing, or no information was available on the patent status of the manufacturing procedure.

For the purposes of these experiments, samples of the 33 vaccines were purchased directly from the manufacturers with the specification that they be from the latest production lots.

Fifteen of the vaccine products were of lapine origin and are coded in this report by letters in alphabetical sequence from A to I, inclusive, and from \(BB\) to \(GG\), inclusive. There were five vaccine products of porcine origin which are designated in the same way by the letters \(J\) to \(N\), inclusive. The tissue culture vaccine products were 13 in number and are identified by the letters \(O\) to \(Z\), inclusive, and \(AA\).

\textit{Antiserum}.—The antiserum used in this work was a commercial HC hyperimmune antiserum. The entire production of one serial (s.n. 1658) was purchased from the Rea Serum Co., Tallahassee, Florida, in 1956. Titration of this antiserum indicated that \(0.5\) ml./lb. of live pig weight imparted passive immunity to pigs against 44,000 LD\textsubscript{50} doses of hog
cholera virus for 21 days, but similar serum-treated pigs were again susceptible at 28 days after injection.

**Challenge Virus.**—The challenge virus was the second pig passage from Bureau of Animal Industry regular, virulent HC virus 7183 (1946) presently identified in other publications as the Ames strain of virulent challenge virus. It was identified and will be referred to in this paper as Station Hog Cholera Virus (SHCV).

The LD$_{50}$ titer of SHCV was determined to be $10^{4.64}$ or 43,650, about 44,000 LD$_{50}$ doses/ml. by the Reed-Muench$^{10}$ method on the basis of titrations made with 35 Florida and Georgia farm source pigs. The titer of the virus in Georgia pigs was $10^{4.45}$ and in Florida pigs it was $10^{4.77}$.

**Experimental Animals.**—One group of the susceptible pigs used in the experiments and titrations was obtained from two source herds, one in Lowndes County, Georgia, and one in Suwannee County, Florida. There was no past history of HC on either farm. In addition, the owner of the Lowndes County, Georgia, herd had not used modified live virus vaccine for at least two years. The owner of the Suwannee County, Florida, swine herd reported that he had never used modified live virus vaccine at any time. The pigs from both herds were predominantly grade Hampshire. Pigs from each source were used in approximately equal numbers for all vaccine tests as well as for the serum and virus controls and virus titrations.

The other group of susceptible pigs for the tests and controls was hysterectomy-derived SPF pigs and second generation SPF pigs provided by Animal Services of the National Animal Disease Laboratory, Ames, Iowa. These pigs were either grade Yorkshires, Chester Whites or Hampshires.

One hundred thirty-two pigs were used as vaccinated principals. One hundred thirty-two pigs were used as contact principals and 66 pigs were used as contact principal alternates. Fifty-four pigs were used as controls. Thirty-five pigs were used to titrate SHCV. The total number of pigs used, therefore, was 419.

**METHOD**

The work being reported here was originally designed to be carried out in the field and was begun on a number of cooperating farms. The experiment as then designed required the use of modified live virus vaccines, without antiserum on some farms, as well as with the simultaneous administration of antiserum on other farms and leaving a specified number of nonvaccinated pigs on all farms to measure virus spread. Pairs of vaccinated pigs and nonvaccinated contact pigs were purchased from each farm for challenge on the day before the pigs were to be brought to market. This was done to avoid exposure of the pigs on the market premises. The pigs were brought to the Hog Cholera Research Station in Florida and challenged by exposure to 44,000 LD$_{50}$ doses of SHCV. This challenge revealed no evidence of transmission of
virus and it was realized that the massive challenge dose was obliterating the manifestation of any measurable transmission of virus from the vaccinated pigs to the contact pigs. As time went on, it became more and more difficult to recruit cooperative farmers in the program because of their fear of potential hog cholera outbreaks in association with the use of the vaccines without antiserum. Finally, because of the failure to demonstrate positive results of transmission of virus and the lack of cooperation, this form of the experiment was canceled.

The experiment was then redesigned to be performed entirely on the premises of the Hog Cholera Research Station in Live Oak, Florida, and the National Animal Disease Laboratory, Ames, Iowa. There were seven testing periods with from three to six products testing during each period. This is shown in Table I. The facilities at the Hog Cholera Research Station were such that strict isolation could be maintained for a single treatment group of five pigs or for single pigs. All pigs were observed twice daily and appetite, attitude, and other clinical signs were recorded, but no temperatures were taken in order to preclude accidental transmission of virus.

A typical test of one vaccine in its redesigned form required 10 pigs plus controls. These were treated as follows:

Day 0 - Vaccinates.—Two pigs were injected with the vaccine under test simultaneously with 0.25 ml. of antiserum/lb. of live body weight. Two other pigs were penned separately and injected with the same test vaccine but without antiserum.

Antiserum Controls.—Two or three more pigs were injected with the same dose of antiserum and kept in isolation.

Two Days Post-Vaccination (DPV) - Contacts and Alternate Contacts.—Three pigs were placed in contact with each of the pairs of vaccinates. Two of each group of three were to be challenged 28 days later with 4.4 LD50 doses of SHCV. This was 1.0 ml. of a 10^-4 dilution of SHCV. The third pig was intended as an alternate in the event of death before challenge from virus transmission, bleeding or other causes.

<table>
<thead>
<tr>
<th>Date of experiment</th>
<th>Code letters</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/11/64</td>
<td>A, B, C, D</td>
<td>Lapine origin</td>
</tr>
<tr>
<td>11/23/64</td>
<td>E, F, G, H, I</td>
<td>Lapine origin</td>
</tr>
<tr>
<td>10/5/65</td>
<td>BB, CC, DD</td>
<td>Lapine origin</td>
</tr>
<tr>
<td>12/21/65</td>
<td>EE, FF, GG</td>
<td>Lapine origin</td>
</tr>
<tr>
<td>1/5/65</td>
<td>J, K, L, M, N</td>
<td>Porcine origin</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>Tissue culture</td>
</tr>
<tr>
<td>1/26/65</td>
<td>O, P, Q, R, S, T</td>
<td>Tissue culture</td>
</tr>
<tr>
<td>3/2/65</td>
<td>V, W, X, Y, Z, AA</td>
<td>Tissue culture</td>
</tr>
</tbody>
</table>
30 DPV - Vaccinates.—The two pairs of vaccinates were challenged with 44,000 LD₅₀ doses of SHCV.

30 DPV - Antiserum Controls.—The two or three antiserum control pigs were also challenged with 44,000 LD₅₀ doses of SHCV.

28 Days Post Contact (DPC) - Contacts and Alternate Contacts.—The two contact pigs or alternates were each removed from the contact pen, penned separately in an isolation pen and challenged with 4.4 LD₅₀ doses of SHCV.

4.4 LD₅₀ Controls.—Two or three previously untreated pigs were also penned separately and in isolation and injected with 4.4 LD₅₀ doses of SHCV as controls.

51 DPV (21 days after the 4.4 LD₅₀ challenge) - Contacts.—The survivors of the 4.4 LD₅₀ dose challenge were then challenged with 44,000 LD₅₀ doses of SHCV.

44,000 LD₅₀ Controls.—Two or three more untreated pigs were also injected with this dose of virus as controls.

Transmitted vaccine virus is characterized by the terms, lethal HCV and immunizing HCV. The use of these terms is merely a convenient means of designating the characteristics of the transmitted virus. Their use should not be interpreted to mean that they represent different viruses but rather different characteristics of the same virus.

Determination of the transmission of lethal HCV was made on the basis of a presumptive diagnosis of HC. Criteria for the diagnosis were clinical signs, death and lesions at necropsy usually seen in HC. Confirmation was made by the fluorescent antibody tissue culture test for HC as described by Mengeling et al.¹¹

Determination of the transmission of immunizing HCV was made on the basis of survival of the contact pig after virus-challenge. To accomplish this, two challenges of these contact pigs were made. The first challenge dose was 4.4 LD₅₀ doses of SHCV. Twenty-one days later, the survivors of the first challenge were injected with 44,000 LD₅₀ doses of SHCV as described above.

The rationale of using a dilute challenge dose initially was based on the work of Zinober.¹² In this work it was demonstrated that a low level of immunity against HC could be obtained in pigs with small sublethal doses of virulent HCV which would not withstand challenge against several thousand lethal doses of virulent virus but would protect pigs and could be measured by challenge with a dosage containing a low number of lethal doses of the same challenge virus. This diluted virus challenge was therefore selected to provide a more sensitive test of transmission of vaccine virus from vaccinated to non-vaccinated pigs. A first challenge dose of 4.4 LD₅₀ doses of virus was considered to contain sufficient virus to cause death and not overwhelm minimal immunity by a massive challenge of virus. In other words, such a sensitive challenge dose could be used where critical determinations were to be made. Since the work being reported here was precisely of this nature, it was decided to use a 4.4 LD₅₀
virus challenge dose initially, followed by a 44,000 LD$_{50}$ challenge
dose to the survivors.

Although the 4.4 LD$_{50}$ challenge prior to the 44,000 LD$_{50}$ chal-
lenge may have had a potentially immunizing effect, this larger dose
was used to demonstrate that this massive dose could not be used to
detect transmission of immunizing virus. Theoretically, if some pigs
did not react after exposure to the 4.4 LD$_{50}$ challenge and were still
susceptible when challenged with the larger 44,000 LD$_{50}$ dose, it would
demonstrate the overwhelming effect of the larger virus dose. In the
face of this overwhelming effect, it would therefore not be possible to
detect the transmission of minimal quantities of immunizing virus by
contact.

RESULTS

Lapine Origin Vaccines

Vaccinates.—None of the 18 pigs vaccinated with nine lapine origin
vaccines simultaneously with antiserum or the 18 pigs vaccinated with
the same products without antiserum exhibited any clinical signs of
HC following vaccination and all were immune following challenge 30
DPV with 44,000 LD$_{50}$ doses of SHCV.

Contacts.—Twelve of 15 lapine origin vaccines, 80.0 percent,
transmitted lethal HCV and/or immunizing HCV to 37 of 56 contact
pigs, 66.1 percent.

Four of 15 lapine origin vaccines, A, E, F and H, Tables II and
III, transmitted lethal HCV to 12 of 18 contacts. One vaccine, F,
transmitted lethal HCV to two of three pigs only when used with anti-
serum; one vaccine, E, transmitted lethal HCV to two or three pigs
only when used without antiserum and two vaccines, A and H, trans-
mitted lethal HCV to eight of 12 pigs when they were used simultaneously
with antiserum and also when they were used without antiserum.

Eleven of 15 lapine origin vaccines, A, B, C, D, E, F, CC, DD, EE,
FF and GG, Table I, transmitted immunizing HCV to 25 of 38 contacts,
as indicated by survival after challenge with 4.4 LD$_{50}$ doses of SHCV,
Tables II and IV. Seven of the 25 survivors then withstood the subsequent
challenge with 44,000 LD$_{50}$ doses of SHCV and the remaining 18 pigs
died with signs and lesions at necropsy usually associated with hog
cholera.

Three of the vaccines mentioned above, A, B and C, transmitted
immunizing HCV to three of eight pigs when they were used simulta-
neously with antiserum; two, D and E, to two of six pigs when they
were used without antiserum; and six, F, CC, DD, EE, FF and GG, to
20 of 24 pigs when they were used both with and without antiserum.

It should be noted that 11 of 15 lapine origin vaccines transmitted
immunizing HCV, three of which also transmitted lethal HCV and one
vaccine transmitted lethal HCV only.

Three lapine origin vaccines, G, I and BB, were negative for trans-
mission of virus to contact pigs.
### TABLE II
Contact Transmission of Lethal and/or Immunizing Virus by Modified Live Hog Cholera Virus Vaccines When Used with and/or without Antiserum

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Negative for transmission</th>
<th>Lethal Virus</th>
<th>Immunizing virus only</th>
<th>Total positive transmission vaccines of all types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative vaccines</td>
<td>With and without antiserum</td>
<td>With antiserum</td>
<td>Without antiserum</td>
</tr>
<tr>
<td></td>
<td>Tissue culture origin</td>
<td>Non-immunizing</td>
<td>With &amp; without antiserum</td>
<td>With &amp; without antiserum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lapine origin</td>
<td>G,I,BB</td>
<td>H</td>
<td>A</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>3/15</td>
<td>20.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>J†,M†, N†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V,W,Y</td>
<td>U†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/13</td>
<td>30.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>7</td>
<td>7/33</td>
<td>21.2</td>
<td>9/33</td>
</tr>
</tbody>
</table>

* numerator = negative; denominator = studied.
** Numerator = positive; denominator = number of vaccines studied.
† Also transmitted lethal virus to vaccinated pigs.
‡ This vaccine caused a severe reaction in one vaccinate, but the pig recovered and was considered immune after challenge with 44,000 LD50 doses of virus.
<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>With antiserum</th>
<th>Without antiserum</th>
<th>With and without antiserum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccines</td>
<td>Pigs</td>
<td>Vaccines</td>
<td>Pigs</td>
</tr>
<tr>
<td></td>
<td>No*</td>
<td>%</td>
<td>No*</td>
<td>%</td>
</tr>
<tr>
<td>Lapine origin</td>
<td>1/15 6.7</td>
<td>2/3 66.7</td>
<td>1/15 6.7</td>
<td>2/3 66.7</td>
</tr>
<tr>
<td></td>
<td>2/15 13.3</td>
<td>8/12 66.7</td>
<td>4/15 26.7</td>
<td>12/18 66.7</td>
</tr>
<tr>
<td>Porcine origin</td>
<td>0/5</td>
<td>-</td>
<td>0/5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3/5 60.0</td>
<td>7/12 58.3</td>
<td>3/5 60.0</td>
<td>7/12 58.3</td>
</tr>
<tr>
<td>Tissue culture</td>
<td>1/13 7.7</td>
<td>2/3 66.7</td>
<td>0/13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1/13 7.7</td>
<td>3/3 100.0</td>
<td>2/13 15.4</td>
<td>5/6 83.3</td>
</tr>
<tr>
<td>Total</td>
<td>2/33 6.1</td>
<td>4/6 66.7</td>
<td>1/33 3.0</td>
<td>6/33 18.2</td>
</tr>
<tr>
<td></td>
<td>6/33 18.2</td>
<td>18/27 66.7</td>
<td>9/33 27.3</td>
<td>24/36 66.7</td>
</tr>
</tbody>
</table>

*a* Numerator - positive; denominator - studied.

† Numerator - positive; denominator - exposed to positive vaccines.
TABLE IV

Transmission of Immunizing Virus to Contact Pigs from Modified Live Hog Cholera Virus Vaccines as Indicated by Challenge with 4.4 LD50 Doses of Station Hog Cholera Virus

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Vaccine used with and/or without antiserum</th>
<th>With antiserum</th>
<th>Without antiserum</th>
<th>With and without antiserum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccines</td>
<td>Pigs</td>
<td>Vaccines</td>
<td>Pigs</td>
<td>Vaccines</td>
</tr>
<tr>
<td>Lapine origin</td>
<td>3/15</td>
<td>20.0</td>
<td>3/8</td>
<td>37.5</td>
<td>2/15</td>
</tr>
<tr>
<td>Porcine origin</td>
<td>0/5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
</tr>
<tr>
<td>Tissue culture</td>
<td>1/13</td>
<td>7.7</td>
<td>1/1</td>
<td>100.0</td>
<td>1/13</td>
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<tr>
<td>Total</td>
<td>4/13</td>
<td>12.1</td>
<td>4/9</td>
<td>44.4</td>
<td>3/33</td>
</tr>
</tbody>
</table>

*Numerator - positive; denominator - studied.
†Numerator - positive; denominator - exposed to positive vaccine.
Controls.—The results of challenge of all control pigs are given in Table V.

Porcine Origin Vaccines

Vaccinates.—The 10 pigs vaccinated with five vaccines simultaneously with antiserum exhibited no clinical signs of HC and, when they were challenged with 44,000 LD50 doses of SHCV 30 DPV, did not react and were considered to be immune. Of the five vaccines used without antiserum, however, four of them (J, K, M, and N) proved to be lethal by producing clinical signs, death and lesions at necropsy usually associated with HC in four of eight vaccinated pigs. The four pigs which died were all from the Florida farm source.

Contacts.—Five of 5, 100.0 percent porcine origin vaccines transmitted lethal HCV and/or immunizing HCV to 20 of 23 contact pigs, 87.0 percent.

Three of five porcine origin vaccines, J, M, and N, Tables II and III, transmitted lethal HCV to seven of 12 contacts when they were used either with or without antiserum.

All five vaccines studied, J, K, L, M and N, Tables II and IV, transmitted immunizing HCV to 13 of 23 contacts when they were used either with or without antiserum as indicated by survival after challenge with 4.4 LD50 doses of SHCV. Eleven of the 13 survivors also survived a challenge of 44,000 LD50 doses.

None of the porcine origin vaccines were negative for transmission of virus to contact pigs.

Controls.—See Table V for results of challenge of all control pigs.

TABLE V
Challenge of Control Pigs

<table>
<thead>
<tr>
<th>Date of experiment</th>
<th>Type of control</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antiserum</td>
<td>4.4 LD50 of virus</td>
<td>44,000 LD50 of virus</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Number of pigs died/number of pigs challenged</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/11/64</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>6/6</td>
</tr>
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<td>2/2</td>
<td>6/6</td>
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<tr>
<td>10/5/65</td>
<td>3/3</td>
<td>2/3</td>
<td>3/3</td>
<td>8/9</td>
</tr>
<tr>
<td>12/21/65</td>
<td>3/3</td>
<td>2/2</td>
<td>3/3</td>
<td>8/9</td>
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<tr>
<td>1/26/65</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>9/9</td>
</tr>
<tr>
<td>3/2/65</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>9/9</td>
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<td></td>
<td>18/18</td>
<td>16/18</td>
<td>18/18</td>
<td>52/54</td>
</tr>
<tr>
<td>Total</td>
<td>100.0%</td>
<td>88.9%</td>
<td>100.0%</td>
<td>96.3%</td>
</tr>
</tbody>
</table>
Vaccinates.—Of the 13 tissue culture vaccines used simultaneously with antiserum, one of them, vaccine U, was lethal to one of two vaccinates. This pig, which was from the Florida farm source, developed clinical signs, died and had lesions at necropsy usually associated with HC. Another pig vaccinated with a different vaccine, Z, had a severe reaction but recovered. When it was challenged with 44,000 LD$_{50}$ doses of SHCV #30 DPV together with the other 24 vaccinates, they were all negative and were considered to be immune.

When these vaccines were used without antiserum, vaccine U again was lethal to one vaccinate. This pig, which also was from the Florida source, developed clinical signs, died and had lesions at necropsy usually associated with HC. Another pig which had been vaccinated with vaccine O was killed by the other pigs in the pen during the contact period and therefore was not challenged. When these 24 pigs were challenged with 44,000 LD$_{50}$ doses of SHCV #30 DPV, they did not react and were considered to be immune.

Contacts.—Nine of 13 tissue culture vaccines, 69.2 percent, transmitted lethal HCV and/or immunizing HCV to 30 of 35 pigs, 85.7 percent.

Two of the 13 vaccines studied, U and X, Tables II and III, transmitted lethal HCV to five of the six contacts. One vaccine, X, transmitted lethal HCV to two of three pigs only when it was used simultaneously with antiserum. One vaccine, U, transmitted lethal HCV to three of three pigs when it was used with antiserum as well as when it was used without antiserum.

None of the 13 vaccines studied, O, P, Q, R, S, T, U, X and AA, Tables II and IV, transmitted immunizing HCV to 25 of 29 contacts, as indicated by survival from challenge with 4.4 LD$_{50}$ doses of SHCV. Seven of the 25 immune pigs, which survived the 4.4 LD$_{50}$ challenge exposure also survived a 44,000 LD$_{50}$ challenge administered 21 days after the 4.4 LD$_{50}$ exposure. The other 18 pigs died with clinical signs and lesions usually seen in hog cholera. One vaccine, X, when used simultaneously with antiserum, transmitted immunizing HCV to one of one contact pig. One vaccine, AA, when used without antiserum and challenged with 4.4 LD$_{50}$ doses of virus transmitted immunizing HCV to one of one contact pig. Seven vaccines, O, P, Q, R, S, T and U, when used and without serum and challenged with 4.4 LD$_{50}$ doses of virus, transmitted immunizing HCV to 23 of 27 contacts.

Four tissue culture vaccines, V, W, Y and Z, were negative for transmission of virus to contact pigs.

Controls.—The results of challenge of all control pigs are given in Table V.

All Vaccines

Twenty-six of the 33 vaccines, 78.8 percent, studied transmitted lethal and/or immunizing HCV to 87 of 126 pigs, 69.0 percent.
Nine of the 33 vaccines studied, 27.3 percent, Table III, from all three types transmitted lethal HCV to 24 of 36 contacts, 66.7 percent and all but one did so even with the simultaneous use of antiserum. The ranking of the vaccines according to transmission of lethal HCV was: porcine origin, 60.0 percent; lapine origin, 26.7 percent; and tissue culture, 15.4 percent.

Twenty-five of the 33 vaccines studied, 75.8 percent, from all three types transmitted immunizing HCV to 63 of 90 contacts, 70.0 percent, following challenge with 4.4 LD$_{50}$ doses of SHCV. Moreover, 22 of the vaccines, 66.7 percent, did so even with the simultaneous use of antiserum. When the 63 survivors of the 4.4 LD$_{50}$ challenge were challenged with 44,000 LD$_{50}$ doses, 25, 39.7 percent, survived. The ranking of the vaccines according to the transmission of immunizing HCV was: porcine origin, 100 percent; lapine origin, 73.3 percent; tissue culture, 69.2 percent.

Twenty-six of the 33 vaccines studied, 78.8 percent, Table II, from all three types transmitted lethal and/or immunizing HCV to 87 contact pigs. Twenty-three of the 26 vaccines, 88.5 percent, transmitted both forms of HCV to 45 of 65 contact pigs, 69.2 percent, even with the simultaneous use of antiserum. Comparably, 22 of the 26 vaccines, 84.6 percent, transmitted lethal and/or immunizing HCV to 42 of 61 contact pigs, 68.9 percent, when used without antiserum. Nineteen vaccines transmitted both forms of virus when they were used both with and without antiserum. Five of the 33 vaccines, 15.2 percent, also caused death with clinical signs and lesions of HCV in vaccinated pigs.

Differences in reaction were observed among the pigs depending on their geographical source (Florida or Georgia) but no differences were noted in regard to their parturient source (hysterectomy-derived or second generation SPF).

A total of 108 contact pigs were used from Florida and Georgia, 54 from one farm source in each state. Transmission of lethal and/or immunizing HCV was demonstrated by 39, 72.2 percent, of the 54 Georgia pigs and 30, 55.6 percent, of the 54 Florida pigs, Table VI. Fifteen of the 54 Florida pigs, 27.8 percent, died as a result of transmission of lethal HCV, whereas only nine of the 54 Georgia pigs, 16.7 percent, died for this reason. Immunizing HCV was transmitted to 30 of 45 Georgia pigs, 66.7 percent, and to 15 of 39 Florida pigs, 38.5 percent.

A total of 24 SPF contact pigs were used; 12 were hysterectomy-derived and 12 were second generation pigs. Transmission of immunizing HCV was demonstrated by nine pigs, 75.0 percent, of each category, but no lethal HCV was transmitted to any of them.

Eighteen pigs were used for controls of the 4.4 LD$_{50}$ challenge. Twelve of the 18 pigs were from the Florida and Georgia farm sources and six of the 18 were SPF pigs. All 12 of the Florida and Georgia pigs died as a result of this challenge whereas only four of the six SPF control pigs died.
### TABLE VI
Transmission of Hog Cholera Virus to Florida and Georgia Farm Source Pigs

<table>
<thead>
<tr>
<th>Source of pigs</th>
<th>Lethal hog cholera virus</th>
<th>Immunizing hog cholera virus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number*</td>
<td>Percent</td>
<td>Number*</td>
</tr>
<tr>
<td>Florida</td>
<td>15/54</td>
<td>27.8</td>
<td>15/39</td>
</tr>
<tr>
<td>Georgia</td>
<td>9/54</td>
<td>16.7</td>
<td>30/45</td>
</tr>
</tbody>
</table>

*Numerator - number of pigs which demonstrated transmission of virus. Denominator - number of pigs exposed.

### DISCUSSION

**Vaccinates.**—The importance of the source of experimental pigs is demonstrated by the fact that all six of the vaccinates which died were from the Florida farm source. These results are not necessarily alarming since five of these pigs had not received antiserum with the vaccines (four had received porcine origin and one had received tissue culture vaccine). Their mates, however, which were from the Georgia farm source, received the same treatment, i.e., no antiserum with the vaccine, but they survived. This correlates with the titration of the challenge virus in pigs from these two sources which showed the Florida pigs to be more susceptible to HCV than the Georgia pigs.

**Contacts.**—There is real danger in the transmission of lethal HCV, regardless of whether or not antiserum is used with the vaccine. Death losses in the presently described experiments from the transmission of lethal HCV should not be attributed to under-dosage of antiserum, since the dosage used in this work was in accordance with manufacturer antiserum dosage recommendations.

The transmission of immunizing HCV is potentially dangerous because of the enhancement of virulence of such viruses by passage through successive pigs. This has been demonstrated experimentally by Torrey *et al.* and may occur naturally. In this gradual accretion of virulence, so-called mild forms of HC may occur without any ascertainable cause and may finally erupt into typical acute HC.

In the transmission of lethal HCV the greater susceptibility of the Florida over the Georgia pigs is again demonstrated, 27.8 percent and 16.7 percent, respectively, Table VI. This again confirms the titration results, which showed the Florida pigs to be more susceptible to lethal HCV than the Georgia pigs. The transmission of immunizing HCV, however, indicates a greater ability to develop immunity of the Georgia over the Florida pigs, 66.7 percent and 38.5 percent, respectively. This is diametrically opposite to the transmission of lethal HCV. These results indicate that although the Florida pigs were more susceptible to lethal HCV, the greater susceptibility of the Georgia pigs to immunizing HCV indicated a more efficient defense mechanism and a greater ability...
to develop immunity than the Florida pigs. This suggests that susceptibility alone is not necessarily an adequate criterion for determination of optimum subjects for immunizing procedures.

The Georgia pigs were more susceptible to the transmission of lethal and/or immunizing HCV, 72.2 percent, than the Florida pigs, 55.6 percent or a ratio of 1.29:1, respectively. The Georgia pigs, therefore, were more useful for the measurement of transmission of modified live vaccine virus.

The spread of both lethal and immunizing HCV occurred from a majority of vaccines of all types regardless of whether or not antiserum was used. Twenty-three vaccines transmitted both forms of HCV when they were used simultaneously with antiserum and 22 vaccines did so when used alone. This contact transmission, therefore, should be considered a characteristic of modified HCV vaccines and their usefulness in an eradication effort should be circumscribed by these limitations. This is true because the 4.4 LD$_{50}$ virus control experiments demonstrated 88.9 percent lethality for this dose in the susceptible pigs. In other words, if there had been no transmission at all from the vaccinates to the contacts, the 4.4 LD$_{50}$ doses should have killed about 90 percent of the contact pigs. Since this dose failed to kill 75 percent of the contact pigs, and assuming the prior susceptibility of these pigs, this is strong evidence that there must have been transmission of at least a minimal immunizing effect from the vaccinates to the contacts.

The rationale of using 4.4 LD$_{50}$ doses as the initial challenge dose was confirmed by the results. In porcine origin and tissue culture vaccines, used both with and without antiserum, there were pigs which withstood the 4.4 LD$_{50}$ challenge dose but not the 44,000 LD$_{50}$ challenge dose. Previous experimental studies$^{10}$ indicated that if the greater dose of virus had been used, these pigs would have been overwhelmed by the massive challenge and the evidence of transmission of minimal quantities of immunizing virus would have been lost.

Controls - Antiserum.—The deaths of these pigs demonstrated that the passive immunity imparted by the antiserum in the vaccine-antiserum vaccinates had been dissipated and that their survival was due to active immunity.

4.4 LD$_{50}$ - Control Pigs.—The deaths of 16 of 18, 88.9 percent, of these pigs demonstrated that this dose of SHCV was lethal to almost 90 percent of susceptible pigs and that those contact pigs which survived this dose had received an immunizing factor, probably a virus, from the vaccinated pigs with which they had been in contact.

Twelve of these 18 controls were from the Florida and Georgia farm sources and all 12 of them died after receiving this challenge dose. The other six pigs were SPF and only four of them died. This demonstrates the greater susceptibility of these Florida and Georgia control pigs to HCV then the SPF pigs used for controls.

44,000 LD$_{50}$ —The deaths of all control pigs injected with this dose of SHCV demonstrated its lethality and indicated the probable susceptibility of the experimental pigs.
SUMMARY AND CONCLUSIONS

1. Of 33 modified live hog cholera virus vaccines, five of them, 15.2 percent, were lethal to 5 of 10 vaccinates.
2. Nine of the 33 vaccines, 27.3 percent, transmitted lethal hog cholera virus by contact; eight of them did so even with the simultaneous use of antiserum.
3. Twenty-five of the 33 vaccines, 75.8 percent, transmitted immunizing virus by contact; 22 of them did so even with the simultaneous use of antiserum.
4. Eight of the 33 vaccines, 24.2 percent, transmitted both lethal and immunizing virus by contact; seven of them did so even with the simultaneous use of antiserum.
5. Twenty-three of 26 vaccines, 88.5 percent, transmitted lethal and/or immunizing HCV to 45 of 65 contact pigs, 69.2 percent, when used simultaneously with antiserum compared to 22 of 26 vaccines, 84.6 percent, which transmitted both forms of HCV to 42 of 61 contact pigs, 68.9 percent, when used without antiserum.
6. The Florida pigs were more susceptible to lethal HCV, 27.8 percent, than the Georgia pigs, 16.7 percent, whereas more of the Georgia pigs, 66.7 percent, developed immunity than the Florida pigs, 38.4 percent.

REFERENCES


The campaign to eradicate hog cholera has entered a critical period. The earlier efforts of this Association in evaluating the probability of eradication as well as formulating recommendations for cooperative action have formed a solid foundation for the eradication of hog cholera from this country. Hog cholera incidence is at an all-time low and the disease is now exotic to large areas of the country. Major swine-producing States have made substantial gains on hog cholera incidence and have seized the opportunity to advance to the eradication phases of the program.

The critical need at this point is to avoid the tendency to relax program effort now that incidence has been reduced. If such a tendency develops, many of the gains could be lost. A buildup of incidence could occur, and eradication would be even more difficult.

The purpose of this discussion is to review progress to date as well as to note problems with which the program must reckon in the near future.

PROGRAM STATUS

By October 1, 1965, all States but one were engaged in the cooperative program, with 14 States having advanced beyond the control phases (Phase I and II) of the program (Figure 1). By July 1, 1966, only three States remained in Phase I, 26 States and Puerto Rico were in Phase II, 10 in Phase III, and 11 in Phase IV. Of these States in Phase IV, five have qualified as hog cholera free under the standards recommended by this Association. This compares with two which were recognized as hog cholera free in October 1965.

More important than mere numbers of States in advanced stages is the position those States occupy with relation to each other and the swine population. For instance, ten Western States and one North Central State form a large block of States operating in the final phases of the program. While it is recognized that these States generally have small swine populations, their ability to move and to maintain this position, and thereby form a large contingent area operating in advanced phases, is noteworthy.

Considerable progress was made over the year by States with larger swine populations. Wisconsin (eighth in swine population) and Michigan...
Cooperative State-Federal

Hog Cholera Eradication Program

July 1, 1966

Figure 1

(eighteenth) have advanced to Phase IV. These States contain more swine than the total number of swine covered by Phase IV previously.

Probably the most important advance in program status during these months concerns Illinois and Missouri. These States, with 20 percent of the Nation's swine, moved to Phase III as the fiscal year ended.

INCIDENCE OF HOG CHOLERA

Advancement through the various program phases is meaningless unless there is corresponding decline in hog cholera incidence. In fiscal year 1966, 583 of 1432 suspicious outbreaks reported were confirmed (Figure 2). In 1965, 1110 of 1727 reports were confirmed; therefore, hog cholera confirmations declined 47 percent in fiscal year 1966. While this decline is probably due in large part to program activity since 1962, it does not justify conclusions that the program is nearing completion. Nor does the decline justify a conclusion that similar inroads will be made on hog cholera incidence in future years without more intensive program effort. However, the sharp decline in outbreaks does provide a basis for systematic advancement in program effort until hog cholera is eradicated.

Reports of suspected outbreaks declined 18 percent in fiscal year 1966 from 1965. Since the declination of reports is less than one-half the 47 percent decline in confirmed outbreaks, it appears that hog cholera reporting systems are being maintained or improved.

Constant appraisal of reporting systems, as well as methods to improve them, must be maintained so that reporting suspicious illnesses is
OUTBREAKS REPORTED

NUMBER OUTBREAKS

<table>
<thead>
<tr>
<th>Year</th>
<th>Suspicious</th>
<th>Confirmed</th>
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</thead>
<tbody>
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<td>1,558</td>
</tr>
<tr>
<td>1963</td>
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<td>320</td>
<td>373</td>
</tr>
<tr>
<td>1967</td>
<td>467</td>
<td>481</td>
</tr>
</tbody>
</table>

Figure 2

PERCENT OF SUSPICIOUS REPORTS CONFIRMED AS HOG CHOLERA

PERCENT

<table>
<thead>
<tr>
<th>Percent</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
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</tr>
<tr>
<td>50</td>
<td>1966</td>
</tr>
<tr>
<td>41</td>
<td>1967</td>
</tr>
</tbody>
</table>

12 MONTH RUNNING AVERAGE.

Figure 3
at the highest level possible. This need is becoming increasingly apparent as the program advances with concurrent recognition of less obvious forms of the disease.

DIAGNOSIS

The confirmation rate of suspicious outbreaks has steadily declined since the program's beginning. In fiscal year 1964, the first year information regarding confirmed outbreaks was available, 70 percent of the reported outbreaks were confirmed, compared to 41 percent confirmed in 1966 (Figure 3). While this is further evidence that reporting systems have maintained their effectiveness, this should not be regarded as the ultimate need of the eradication program. Judging from British experience, as well as our own, the confirmation rate should be steadily depressed from this point as the program proceeds.

There has been continuous improvement in the use of laboratory support for hog cholera diagnosis (Figure 4). Prior to the program, it was estimated that laboratory examination was utilized in one-third of the outbreaks. This usage increased to 60 percent in 1964, to 80 percent in 1965, and to 86 percent in 1966. A goal of 100 percent is necessary, and the examination must be of sufficient scope to satisfy the diagnostic guidelines adopted by this Association in 1964. At this point in the program, no diagnostic effort should be considered complete until all
available laboratory procedures have been utilized by the field veterinarian before he makes his final determination.

Field diagnosticians report more obscure forms of hog cholera with increasing frequency. Other reports deal with forms of hog cholera not previously recognized under field conditions in this country. It is most doubtful that diagnosis could have been made in these instances without laboratory support. Therefore, it appears that the program is at the point where swine specimens submitted to laboratories for any purpose be subjected to hog cholera diagnostic procedures in order to promptly deal with obscure forms of the disease as they emerge.

EPIDEMIOLOGY

Virtually all of the suspicious reports of hog cholera were investigated by cooperating veterinarians in 1966. Apparent sources of infection were established in 68 percent of the confirmed outbreaks in 1966 compared to 61 percent in 1965. In the three complete years of program activity, the ability to establish sources of infection has doubled in spite of the disease becoming more difficult to recognize. Even so, cooperating epidemiologists could not establish sources of outbreaks in almost one-third of the outbreaks confirmed, although three years of experience with hog cholera epidemiology have been gained. Therefore, epidemiological procedures must keep pace with the program, and the present improvement must not be regarded as adequate to meet future needs.

Hog cholera outbreaks were confirmed in 343 counties in 28 States and Puerto Rico in 1966 (Figure 6), compared to confirmation in 473 counties in 36 States and Puerto Rico in 1965 (Figure 5). No hog cholera was reported in Phase IV States during fiscal year 1966; however, two Phase IV States have experienced a total of three outbreaks in the early months of fiscal year 1967.

Also significant is the fact that there was no dramatic buildup in hog cholera incidence in any area as happened in 1965 in some Southeastern States.

Outbreaks attributed to movement of swine, either intrastate or interstate, decreased in fiscal year 1966 compared to 1964 and 1965 (Figure 7). Outbreaks attributed to area exposure also decreased in 1966. It appears that the shipping rules and quarantine procedures which are a part of Phase II, under which the bulk of the swine population was covered in 1966, are having the desired affect on the spread of hog cholera through shipment of swine. Nevertheless, over one-half of the hog cholera was identified with swine movement or other contact during the year. Efforts must be intensified in order to further reduce this source.

Data furnished by the Veterinary Biologics Division indicates that hog cholera vaccine production declined in 1966, falling about 18 percent from 1965. The Crop Reporting Service estimates that the 1966 pig crop increased about 10 percent in 1966 from that of 1965. Therefore, it appears that a smaller percentage of the swine population was vaccinated in 1966 than was vaccinated in 1965. In spite of this decrease in vaccine production
and availability, hog cholera outbreaks associated with vaccination increased in 1966 with 114 outbreaks compared to 89 in 1965. Of more significance from the standpoint of eradication, vaccination associated outbreaks accounted for 19 percent of all outbreaks confirmed in 1966, which is more than twice the eight percent associated with vaccination in 1965 (see Figure 7).

Anti-hog-cholera serum production and inventory declined in 1966. It appears that serum was not available for use with 24 percent of the modified live virus vaccine produced. This factor may partially account for the increase in vaccine-associated outbreaks.

A review of the available information showed that the porcine origin products had been implicated in a large portion of these outbreaks, and that these products were capable of causing hog cholera in vaccinated as well as in contact swine under controlled conditions. Therefore, action was taken in 1966 to restrict these products from interstate shipment. Field experience and laboratory evidence indicate that further action must be taken to protect the eradication gains from certain vaccines. It becomes increasingly apparent that safety requirements have become paramount to other qualities of a hog cholera vaccine.

During 1966, epidemiology indicated that hog cholera virus could be transmitted from pregnant sows to their fetuses under ordinary swine husbandry, although the sows showed no clinical evidence of hog cholera. The hog cholera virus involved was both field and certain attenuated hog cholera vaccine virus. After such exposure, some sows aborted; others
farrowed malformed or sick, weak pigs; and, others farrowed apparently normal pigs. In several instances, there was apparent transmission of hog cholera virus from the pigs, including those without clinical signs of hog cholera, to other swine on the premises. The first suspicion of hog cholera affecting the herd occurred when the contact swine sickened, and these suspicions were confirmed after laboratory examination.

One should not be dismayed by these findings. The situation appears to require appropriate conditions in the pregnant sow and fetuses before it develops, as it does not appear routinely after the vaccination of pregnant sows. Too, from the eradication program's standpoint, prompt and complete depopulation of infected herds as recommended by this Association severely limits the threat of spread from this source to the remainder of the swine population. Therefore, while extensive epidemiology of hog cholera in this form has not been reported previously from the field in this country, it is apparent that the program as presently designed is adequate to remove this problem.

Hog cholera associated with garbage feeding increased in 1966, accounting for nine percent of the confirmations compared to four percent in 1965 (see Figure 7). This represents a 125 percent increase in this source's proportion of outbreaks. This should not happen, and the buildup emphatically illustrates the need to make appropriate improvements in the inspection procedures, as well as the need to deal appropriately with those garbage feeders involved. This increase in hog cholera associated with garbage feeding also points to potential ineffectiveness in one of the major defenses against the introduction of exotic diseases into this country.

COMMENTS

Substantial gains were made in hog cholera eradication in 1966, but these gains should not be regarded as having been achieved during that year alone. The progress is, in all likelihood, due to the three and one-half years of program activity along guidelines recommended by this Association together with the several years of evaluation which preceded program activity. That the guidelines are sound is amply illustrated by the remarkable decrease in hog cholera incidence, the recognition and effective handling of obscure forms of the disease, and the ability of the cooperating agencies to meet and maintain program standards.

Even though much progress has been made, the program is in one of its most critical periods. In the early days of the program, the emphasis was upon program initiation, and few States were beyond Phase I (Figure 8). Later, emphasis changed and most States moved to Phase II, the maximum control effort.

The current transitional need—from the control procedures in Phase II to the eradication procedures in Phase III or IV—is very likely the most critical need since program initiation. It appears that hog cholera incidence is low enough in all areas that this is no longer a governing factor for this transition. It also appears that this period of low incidence is the
most opportune moment in this country's century of experience with hog cholera to make the transition.

In 1964 this Association's Program and Policy Committee recommended: "...that all funds necessary be provided to adequately maintain an eradication program that will eliminate the economic waste attributable to hog cholera as soon as possible."

There is good opportunity for complacency during this period of low incidence. Whether we can overcome this tendency and grasp this opportunity to make the transition from control to eradication, and make it swiftly, will largely determine our success in eliminating this "economic waste attributable to hog cholera."
REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA


The Committee wishes to express its pleasure that during the year just past, considerable progress has been made in the eradication of hog cholera. We note with pleasure that five states are now declared Free and that only three states are left in the preparatory stage. We are particularly pleased to note that half of the states are now in either Phase III or Phase IV of the program. Insofar as the incidence of cholera is concerned, seven fewer states reported outbreaks during the past year, the number being reduced from 35 states in the previous year to 28, and cholera was confirmed in 130 fewer counties, this figure being reduced to 343 counties from 473 in the previous year. We are pleased also to note that laboratory confirmation for cholera has increased to a remarkable 86 percent of all cases. We, of course, would like to see this accomplished in 100 percent of the cases, but each year it has shown a responsible increase and we look with confidence to see this trend continue.

The Committee notes with concern the increase in the number of cholera outbreaks attributed to or associated with vaccination. The percent of outbreaks in this category is more than double what it was during 1965. There is considerable evidence to suggest that the reporting of this type of outbreak as merely associated with vaccination, or by similar phrasing, is creating some confusion in the minds of the industry and perhaps others, in that many are concluding that this means that all vaccines are unsafe or improperly made or contaminated. In actual fact, the evidence at hand suggests that the overwhelming majority of this type results directly from misuse of the product. There is some evidence that certain of the vaccines may have been responsible for producing cholera even when properly used. All vaccines are under continuous review by the manufacturers, as well as by scientists of the United States Department of Agriculture so that those reaching the market meet all current requirements regarding safety and potency. However, no surveillance can be effective if the recommendations for use of any product, clearly marked on the label and given widespread publicity through many channels of communication, are ignored. The Committee urges continuing effort on the part of the Veterinary Biologics Division of the United States Department of Agriculture to assure that the safety standards of all of these modified live virus products will be continually upgraded so that they will keep pace with the reduction of field virus in this country.
The Committee recommends to the Animal Health Division that where epidemiological evidence indicates that an outbreak of cholera is related to vaccination, that the report be made more specific and the way in which it is related to vaccination be clearly stated insofar as is known, and we further recommend that where such outbreak is attributed to vaccination that the manufacturer of the particular product involved be so informed.

Your Committee wishes again to focus your attention on the matter of pregnant sow vaccination. In a previous report we advised against this practice and all of the manufacturers of the modified live virus vaccines concurred. Additional information regarding the dangers of this practice is available and it appears that there is a certain percentage of the pigs from sows vaccinated during pregnancy with modified live virus vaccine that become carriers and spreaders as a result of exposure to the vaccine virus. Your Committee urges all states to take steps to see that the vaccination of pregnant sows with modified live virus vaccine is discontinued, or where this is known to happen, that such sows be prohibited either intra or interstate movement for other than immediate slaughter purposes. The pigs from such pregnant sows should be considered exposed and dangerous.

The Committee wishes to reaffirm its position that the use of serum alone should continue to be prohibited. For the purposes of moving either within or between states, other than for immediate slaughter, pregnant sows should be allowed to do so if vaccinated prior to pregnancy with serum and modified live virus, or inactivated vaccine, or if such sows were vaccinated during pregnancy with an inactivated vaccine. Such vaccination with an inactivated product should have occurred at least three weeks prior to contemplated movement.

Last year your Committee recommended that a review be made of the standards and regulatory requirements for approved markets. We are pleased to note that such a review has been made in part. We find substantial compliance with the regulations, although there are some deficient areas. There is still some concern, however, whether these standards are sufficient, particularly in light of a total eradication effort. There is in addition considerable evidence to suggest that some of the requirements and some of the definitions are perhaps a little too general in nature and subject to considerable misinterpretation or different interpretations at varying locations. In the national hog cholera eradication program, the Committee feels that this is perhaps one of the more important facets of the effort and we therefore recommend that the Committee on Stockyards, Markets and Transportation be asked to give serious consideration to this problem with the view towards developing specific definitions, standards of construction and operation that both the regulatory forced of the nation and the marketing industry may use as a model, and also for future acceptance by this Association.

You are all aware that after last year's meeting in Lansing, and at the request of several of the mid-western states, your Committee, following a called meeting in Chicago, recommended to the Executive Committee a change in Phase III requirements of the program which would involve
immediate herd depopulation upon hog cholera diagnosis, with permission to market for slaughter without any form of special processing, all apparently healthy swine that were marketable. Following approval by the Executive Committee, recommendation was made to the United States Department of Agriculture and it was immediately made a part of the official program. The Committee felt justified in recommending this change because it was reasoned that this would allow the mid western states involved to come into full Phase III operations as a unit and permit a study of the practice of marketing apparently healthy exposed hogs to see whether or not sufficient virus was being disseminated to represent any threat to the program. Your Committee is quite pleased to note that this study, while of necessity is yet limited in numbers, does suggest that there is little or no danger, at least so far, in this practice. We recommend and urge that this study be continued and enlarged in scope so that we may not only know regarding the virus, but may identify the type and source of swine so involved. This work so far has been confined to spleens and we urge that additional glands also be used and all available history of such swine be furnished along with the results for study.

Your Committee is concerned that following approval of the changes in Phase III for the reasons herein stated, only two of these major swine producing states involved have entered full Phase III operations. The Committee feels that the key to successful early eradication of hog cholera from this nation is the major swine producing states of the Middle West. If we are to break the back of hog cholera infection, efforts must be concentrated in this area wherein lies 60 percent of the nation's hogs. With this accomplished, the Committee is confident the final clean up can be speedily effected. We urge that all concerned, regulatory services both state and national, industry organizations, national, state and local, give immediate attention to this situation and pursue enthusiastically a prompt advancement into a real activist phase in this section.

At the hearing before the Committee on Hog Cholera here this week, some question was raised as to the safety of the inactivated vaccines. There had been some field evidence to suggest that at least one might be associated with hog cholera. A sample of the vaccine in question when tested did not confirm the field evidence. It is noted that three scattered outbreaks were associated with the same serial lot of inactivated vaccine. While the Committee wishes to emphasize that in these isolated instances, this specific serial lot may have been at fault, this should not serve to in any way indict inactivated vaccine products. The Committee does feel, however, and it herein recommends that a thorough re-evaluation of inactivated vaccines be undertaken so that we may be reassured of their safety and value.

With the spread of vesicular exanthema around the country in the early 1950's, the nation began requiring the cooking of all garbage being fed to swine and put this practice under routine surveillance. All garbage feeding was considered potentially dangerous unless the product was thoroughly cooked prior to use, and the amount and kind of surveillance given was the same under all circumstances. This practice has continued
virtually unchanged up to the present and we are currently expending well over $1,500,000 annually to provide surveillance over the feeding of approximately one million swine. The suggestion has been made that consideration be given to the classification of garbage feeding on the basis of type of garbage used and relating this to the number, kind and frequency of inspections required. Your Committee finds much merit in this suggestion and feels that a thorough study and re-evaluation of this entire garbage feeding and inspection operation should be made with a view to recommending and implementing whatever changes modern day practices and conditions may require. The Committee recommends that this Association go on record as urging the United States Department of Agriculture to speedily undertake this effort.
NITRATE-NITRITE INTOXICATION IN SWINE

Terrence M. Curtin, D.V.M., Ph.D.* and
William T. London, D.V.M., M.S.**

Nitrate toxicity has been recognized as a potential hazard to livestock production and human life for more than 100 years. Since the end of World War II, the problem has received more attention due to the greater use of commercial nitrogen fertilizers to increase crop yields, and a higher level of nitrates are in the soil, feeds, and water. In addition, increased concentrations of animal populations have magnified the problem of the disposition of animal manure and other wastes. These materials are a potential source of nitrate contamination of our feed and water supplies.

This report discusses some reported experimental and naturally occurring nitrite intoxication of swine, and an investigation of nitrate-nitrite contamination of water in Missouri. In addition, it describes an experiment conducted to examine effects of chronic exposure of pigs to sublethal doses of potassium nitrite.

HISTORY AND LITERATURE REVIEW

The report in 1946 by Gwatkin and Plummer was among the earliest descriptions of experimental nitrate-nitrite poisoning in swine. Pigs were fasted overnight and administered an oral dose of about one gram of KNO₃ per pound of body weight. The pigs died within 24 hours, but methemoglobinemia was not observed. The stomachs of the pigs were found to be hemmorhagic and edematous, and the contents were tinged with blood. Other gross lesions were not found. These authors suggested the deaths were due to severe gastritis. They also killed fasted swine weighing 20-45 pounds with an oral dose of KNO₃ (5 gms. KNO₃/pig). Death from methemoglobinemia occurred 65 minutes after treatment. No deaths occurred when the trial with KNO₃ was repeated with unfasted pigs. They reported that lesions were not observed in the stomach of pigs given nitrite.

Winks and co-workers investigated deaths on two farms on which swine were fed beef and offal cooked in well water. Chemical analysis of two samples of the feed showed they contained 1127 and 393.6 p.p.m. sodium nitrite. They conducted experiments with swine in which oral doses of 105-124 mg. of sodium nitrite per kg. of body weight produced deaths in pigs within 40-63 minutes. The degree of methemoglobinemia correlated directly with the size of dose administered. However, the amount of

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hemoglobin oxidized to methemoglobin varied between pigs. These authors found, as did Gwatkin and Plummer,\(^8\) that fasted swine are more readily poisoned with nitrite than unfasted swine. They suggested that food dilutes and delays absorption of the nitrite. Winks\(^15\) recommended the stomach contents as the most suitable specimen for use in the diagnosis of nitrite poisoning.

Wanntrop and Swahn\(^15\) pointed out that nitrate is used as a preservative in the cheese industry. During the cheese making process bacteria may reduce nitrate to nitrite, and nitrite poisoning may occur if the whey is fed to swine. In an experiment, they found that the LD\(_{50}\) for swine was somewhat lower than 90 mg. of sodium nitrate per kg. of body weight. Oral administration of nitrite to the experimental pigs produced deaths in 50 to 115 minutes. The maximum methemoglobin levels were reached 90 to 120 minutes post-treatment, and decreased slowly in surviving pigs until after eight to 10 hours it was not detectable.

In Norway, sodium nitrate is used as a preservative for herring, and residues remain in the herring meal used in swine rations. Hvidsten\(^9\) reported no effect on the state of health, feed efficiency or the quality of pork when herring meal containing 10 times the amount of nitrite permitted in herring meal in Norway was fed to 56 pigs. Each pig received 200 grams per day containing up to two percent nitrite. Formation of methemoglobin was not observed.

Garner et al.\(^5\) reported that two percent KNO\(_3\) in the ration of pregnant sows (initiated on the 35th day of gestation), had no effect on gestation or subsequent lactation, and litter size was not affected. However, weakness was noted in some pigs, and in one litter there was evidence of vitamin A deficiency.

Emmerick\(^4\) administered sodium nitrite intravenously to six pigs at one week of age. The dosage was 30 mg./kg. of body weight, and no deaths occurred. However, when these same pigs were given the same dosage at three months of age, one pig died. The five remaining pigs were again treated at 5-1/2 months, and another pig died. The average conversion of hemoglobin to methemoglobin was 32 percent at one week of age, and 69 percent at three months and 81 percent at 5-1/2 months. The explanation offered for the apparently greater susceptibility of older pigs to nitrite was the slower rate of methemoglobin reduction.

Tollett\(^14\) reported that diets containing nitrate greater than 1.84 percent significantly reduced gains in swine. He did not include data on amounts of feed consumed. However, reduced rates of growth are not common in animals receiving nitrates unless accompanied by reduced feed.

Smith et al.\(^13\) reported two instances of nitrite poisoning in swine that had eaten wet oats straw bedding. A laboratory analysis of the oat straw bedding proved that both nitrate and nitrite were present. The authors suggest that reduction from nitrate to nitrite may occur outside the animal's body when moisture and temperature are satisfactory for bacterial action.

In ruminants, the rumen microflora convert the relatively non-toxic
nitrate to toxic nitrite. The livers of all animals and the straited muscles of rats and guines pigs can also effect the conversion. In addition, some transformation occurs in the intestinal tract of most species.

Poisoning in swine results primarily from the ingestion of pre-formed nitrites. The principle sources of nitrate-nitrite toxicity in swine are: accidental ingestion of nitrate-nitrite containing compounds and water contaminated by nitrate-nitrites.

The use of chemical nitrogen fertilizers has increased rapidly during the past two decades, and its use is still growing. This soil additive now accounts for conservatively more than 30 percent of our agricultural crop production. Nitrogen is essential for plant growth, and most is absorbed from the soil as nitrate. In some plants, nitrate is converted to protein in the roots, whereas in other species this transformation occurs in the leaves. High nitrate content of forages may be found after improper or excessive nitrogen fertilization, or when dormth, cloudy weather or some other adverse condition prevents the plant enzymes from converting the nitrate nitrogen into protein. Little nitrate has been found in grains.

NITRATE-NITRITE FOUND IN MISSOURI WATER

The quantity of nitrate or nitrite recently found in many water supplies is too large to be explained by contamination with commercial fertilizers. The analysis of 5000 water samples in Missouri for nitrate and nitrite in 45 counties shows that over 42 percent contained over 5 p.p.m. of nitrate nitrogen. The highest contamination was found in areas with the largest livestock populations. In the northern counties pervious soils overlie low permeable glacial clays, and water accumulates at the junction of these two materials. In this region, water from artesian and deep wells is too salty for domestic use, necessitating that most rural water supplies be from wells 15 to 50 feet deep. This area has been farmed for 75 to 150 years, and livestock production is the main source of income. Many of the farm water supplies are located close to feedlots or silos, and there is a high degree of correlation between the concentrations of nitrates and nitrites in these wells and their proximity to livestock feeding areas.

Soil samples taken from one site to a depth of 23 feet contained a calculated 4600 pounds of nitrate nitrogen per acre. The amount of nitrate in these samples is of particular interest as no livestock had been in the area of the samples for a number of years and there was considerable overgrowth of vegetation over the surrounding area. In addition, no chemical fertilizer was used within 400 yards of the well. A pond on the farm which received drainage from a heavily fertilized corn field was negative for nitrates when samples were collected a number of times during the year. From the high nitrate content still found in these and other deep soil samples where livestock had been fed for years, it appears that once nitrate moves to more than five feet below the surface it is preserved.

In Cooper county (Missouri) soil is productive and there is extensive cattle feeding and swine production. Three hundred seventy-one samples of water supplies were sampled during the spring of 1964. Fifty percent
of the drilled wells, 65 percent of the cisterns, 85 percent of the dug wells and over 80 percent of the springs contained at least five p.p.m. of nitrogen as NO$_3$.

The southern part of the state does not have as many wells containing nitrates and the concentrations are not as high as found in northern Missouri. Livestock production is not as extensive in many areas of southern Missouri, and the use of chemical nitrogen fertilizers is much less than in other areas of the state. However numerous drilled wells from 100 to 300 feet deep contained nitrates. It is believed that in this area the leaching of nitrates from manure at the surface may be carried into the underground water sources. No instances were found in this large area of gravelly soils where nitrates in water could be traced to chemical nitrogen fertilizer applied to crops or to contamination from fertilizer storage.

Samples of pond water contained very low concentrations of nitrate even though they received run-off from feeding areas or pastures. This is to be expected since aquatic plants require nitrogen for growth and remove nitrate ions from the pond water.

**EXPERIMENTAL NITRITE INTOXICATION**

Two basic experiments were conducted to (1) establish NO$_2$ levels sufficient to produce an acute lethal toxicity, and (2) to observe chronic nitrite intoxication of swine. Wanntorp and Swahn$^{15}$ reported the LD$_{50}$ for nitrite in swine was "somewhat less than 90 mg./kg. of body weight." It was necessary to establish a more precise basis for the administration of sublethal amounts for the chronic study.

**Acute Nitrite Toxicity**

Twenty-one test pigs which weighed between 0.3 and 30.5 kg. were divided into three lots. All pigs that received oral doses up to 19.32 mg. NO$_2$-N/kg. survived, and those that received doses of 21.35 mg. or greater of NO$_2$-N/kg. died within 90 to 150 minutes post-treatment.

Clinical signs were absent in pigs that received 6.10 mg. or less of NO$_2$-N/kg. of body weight. The others developed signs which varied in severity with the dose of nitrite. Early signs were; restlessness, frequent urination, and vomiting. Mild dyspnea and cyanosis developed within 90 minutes. In animals that received lethal doses of nitrite, dyspnea and cyanosis were followed by coma and death. Methemoglobinemia was the only observable variation from normal at the time of necropsy. It was determined that 75 percent to 82 percent of the hemoglobin was oxidized to methemoglobin in the pigs that died.

It was therefore established that under the conditions of this experiment, the mg./kg. dosage of NO$_2$-N for the LD$_{0}$ = 19.82 mg. and the LD$_{100}$ = 21.35 mg.

**Chronic Nitrite Toxicity**

A dosage of 18.30 mg. NO$_2$-N/kg. was selected as the maximum daily dosage for use in the chronic study. Forty pigs were divided into six
lots—six for each of five treatments and 10 as controls (Table I). Group I received the maximum daily dosage of 18.30 mg. per kg. of body weight per pig, and Group V received the lowest daily dose of 3.05 mg. per kg. of NO₂-N. Groups II through V received diminishing gradations of nitrate.

The nitrite was administered in drinking water and the amounts of KNO₃ added were dependent upon the average weight of the pigs and the daily water consumption of each group.

A chemical analysis of the feed and fresh water available to the pigs was conducted at the initiation of and at three periods during the trial (Table II). The values were low and not considered to appreciably affect the total intake of nitrate-nitrite and were not included in the calculations of the dosages of nitrites given to each group of pigs.

The performance of the animals is illustrated (Table III). Groups IV and V are omitted for brevity, but their performance was not appreciably different than the control group. The average weight gained per pig in Group III exceeded that of all other groups even though they received 9.15 mg. NO₂-N/kg. per day. Other investigators have reported similar growth stimulations in experimental pigs receiving nitrate-nitrite.

Although Group I, which received an average of 1455 mg.NO₂-N* per pig per day at the end of the trial gained 21.4 kg. per pig less than the

### TABLE I

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Pigs/Group</th>
<th>Mg. NO₂-/kg. of Body Weight</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>18.3</td>
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<tr>
<td>II</td>
<td>6</td>
<td>12.2</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>9.15</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>6.1</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>3.05</td>
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<td>VI</td>
<td>10</td>
<td>Controls</td>
</tr>
</tbody>
</table>

†Administered as an oral KNO₂ equivalent.

### TABLE II

Nitrate Analysis of Feed and Fresh Water for Pigs on Chronic Nitrite Study

<table>
<thead>
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<th>Day of Trial</th>
<th>-1</th>
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<th>60</th>
<th>110</th>
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<td>4.2</td>
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<td>4.5</td>
<td>5.0</td>
<td>4.9</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**ppm.

*88.17 grams KNO₂.
controls, they ate less feed. Group III consumed the most feed, but feed efficiency was comparable to the control group.

The administration of 18.30 mg./kg. of nitrite proved to be just below an LD50. Pig number five (Group I) died nine days after the chronic trials were started. The cause of death was nitrite intoxication, indicated by an 83 percent conversion of its hemoglobin to methemoglobin. No other lesions were observed at necropsy. It was believed that this animal consumed more water at one time than was calculated to contain the sublethal amount of nitrite.

The remaining pigs in Group I developed signs of hypoxia soon after drinking the water with the high levels of nitrite. The signs were present for one to two hours, after which they appeared normal. These signs would reappear each time they drank the water. Methemoglobin levels during the periods of hypoxia ranged from 40 percent to 68 percent of the total hemoglobin. These signs were not observed in the other treatments and were less severe in Group I as the trial progressed. Methemoglobin levels rarely exceeded 35 percent in the final portion of the experiment.

Methemoglobinemia was considered to be of questionable value as a diagnostic test for nitrate toxicity. Low dosages of nitrite failed to elicit a measurable amount of methemoglobin formation. Higher levels, ranging from 12.20 to 21.35 mg./kg., caused a peak methemoglobin response in 90 to 120 minutes. Animals either died at the time of the highest methemoglobin levels or methemoglobin levels decreased to near zero in eight to 10 hours.

A satisfactory correlation could not be made between the amount of methemoglobin produced in an animal and death of that animal. It was observed that methemoglobin deteriorated in blood samples at a constant rate of 20 percent for every 24 hours held under refrigeration.

Pig number six (Group I) broke its leg on the 70th day. It was removed from the trial and the limb surgically repaired with the use of an intramedullary pin. Four days later it was returned to the group, then receiving 1108 mg. of nitrite per pig. It died six hours later. Sixty-four
percent of the hemoglobin had been oxidized to methemoglobin. Goodman and Gilman\(^6\) report that tolerance to nitrite develops in human cardiac patients receiving nitroglycerin in two to three weeks, and that the tolerance diminishes in about one week after medication is withheld. It appears that tolerance was developed in pig number six, but was decreased during the short post-surgical convalescence period. Death probably resulted from a combination of the vasodilatory effects of nitrite and the methemoglobinemia.

The mechanism of tolerance is unknown, but it was observed (Figure 1) that a compensatory erythrocytosis developed as illustrated by the hyperhemoglobinemia developed in Group I. Hypoxia is a strong stimulus for erythropoiesis.

![Figure 1](image1.png)

Figure 1. Summary of Mean Leukocyte, Lymphocyte and Neutrophil Counts of Pigs with Highest Dosage* of KNO\(_3\) Compared to Control Pigs.

*18.30 mg./kg. of body weight NO\(_2\)-N equivalent.

In addition, a lymphocytic leukocytosis developed, (Figure 2) in all animals that received nitrite. The leukocyte counts and degree of lymphocytosis correlated directly with the level of nitrite consumed. Yoffey \textit{et al.}\(^7\) reported that hypoxia stimulates a marked increase in bone marrow lymphocytes. They suggested that young lymphocytes can serve as stem cells for the formation of erythrocytes.

![Figure 2](image2.png)

Figure 2. Summary of Mean Hemoglobin Levels of Pigs with Highest Dose* of KNO\(_3\) Compared to Control Pigs.

*18.30 mg./kg. of body weight NO\(_2\)-N equivalent.
Histopathology

Liver. Diffuse subcapsular fibrotic areas consistent with the migration of *Ascaris lumbricoides suis* larvae were present in all livers. Lobular fibrosis was prominent, and an eosinophilic infiltration of the parenchyma was pronounced. There was no indication of centrilobular necrosis.

Thyroid Gland. Hyperplasia of the acinar epithelial cells was absent in all pigs.

Kidney. Renal parenchyma was normal for pigs, and squamous metaplasia did not occur in the epithelium covering the renal papillae.

Stomach and Duodenum. The stomachs of all pigs (control and treated) exhibited mild cattarrhal enteritis. The mucosa was infiltrated with lymphocytes and eosinophils.

The lesions described in the literature\(^2\) of the thyroid and of squamous metaplasia of columnar and cuboidal epithelium relative to nitrate-nitrite induced vitamin A deficiency were lacking. In addition, radiographs of the right forelimbs of all pigs were taken at the initiation and termination of the chronic trials. Vitamin A levels were never sufficiently low to produce detectable bone changes. The pigs in Group I showed a gradual but progressive decline in serum vitamin A levels.

Olson *et al.*\(^{11}\) observed that the provitamin, carotene, was destroyed in the stomachs of monogastric animals when nitrite was present. They\(^3\) were unable to demonstrate decreased absorption of vitamin A per pound of feed and therefore animals were not dependent upon the presence of the provitamin. The nitrite received by the pigs did not destroy enough of the vitamin A in the gastrointestinal tract to precipitate deficiency symptoms and lesions.

SUMMARY AND CONCLUSIONS

Nitrite poisoning in swine may result from the accidental ingestion of nitrite containing chemicals, from bacterial reduction of nitrate to nitrite in feeds when suitable moisture and temperature conditions exist, or from nitrite contaminated water. Fasted animals are most susceptible to acute nitrite intoxication.

Deaths from the intoxication are caused by the combined vasodilatory activity of nitrite and the oxidation of hemoglobin to methemoglobin. Deaths from methemoglobinemia occurred when 76 percent to 88 percent of the hemoglobin was oxidized. The maximum methemoglobinemia occurred 90 to 150 minutes after oral administration of potassium nitrite.

The determination of methemoglobin is not considered to be a satisfactory diagnostic procedure for chronic nitrite poisoning. It was observed that methemoglobin deteriorated in blood samples at a constant rate of 20 percent for every 24 hours held under refrigeration.

The levels of nitrate-nitrite found in Missouri water supplies correlated closely with livestock production and the proximity of wells to livestock feeding areas. The application or storage of chemical nitrogen fertilizers was not traced to contaminated water. Manure is considered
to be the principle source of nitrate-nitrite contamination in Missouri water supplies.

An oral dosage of 19.32 mg. NO₂-N/kg. of both weight caused no deaths whereas, an oral dosage of 21.35 mg. NO₂-N/kg. produced 100 percent deaths within 90 to 150 minutes of administration. Therefore, the LD₅₀ under the conditions of this experiment was between 19.32 and 21.35 mg. of nitrite per kilogram of body weight.

Some tolerance develops to nitrite, but diminishes rapidly when nitrite is withheld. An erythrocytosis and a lymphocytic leukocytosis accompanies chronic exposure to nitrates.

The administrations of nitrite to experimental pigs did not depress growth, cause thyroid dysfunction, nor produce clinical nor microscopic signs consistent with vitamin A deficiency.

From the results of the experiment reported, it is concluded that pigs can tolerate oral levels of NO₂-N as KNO₂ equivalent up to 18.30 mg./kg. of body weight over long periods of time without serious effects.

REFERENCES

INTRODUCTION

The garbage fed hog has long been considered the primary source of human trichinosis in the United States. Because of increasing interest in the last few years for the eradication or control of trichinosis, it was felt desirable to conduct a survey of the trichinae incidence in garbage fed swine. It was hoped that incidence information derived from the survey would be helpful in pointing the way toward the type and stringency of controls needed to help eradicate the parasite from this important sector of the United States swine industry.

The survey was concerned with known garbage fed herds coming under the States regulatory authority. This, in general, encompasses all swine herds where garbage is brought onto the premises and garbage is the primary diet.

A most important feature of this survey which differed from previous work along the same lines is that the survey was statistically designed. Through the process of random selection, every known garbage fed herd in every State in the continental United States had the possibility of being sampled. Infected herds did not have a greater chance of being sampled than noninfected herds. Herds with a history of some raw garbage feeding did not have a greater chance of being sampled than cooked garbage herds. Hence, the incidence is indicative of the entire garbage feeding industry.

In addition to collecting diaphragm specimens for determining trichinae infection, blood samples were collected from over 5,000 of these animals. Sera from these samples, identified with their respective diaphragms, have been frozen and are stored by the United States Department of Agriculture's Animal Disease and Parasite Research Division at Beltsville, Maryland. The sera samples will be available for use in any future research to develop a serological test to detect trichinae in the living animal. A very brief farm history as to source of pigs, rodent infestation, adequacy of cooking, etc., was taken for each premises sampled in the hope of learning a common factor, or factors that might be present on all infected premises.

The survey began in July 1964 and was completed in July 1966. Fifteen States participated. There were 5,955 samples from 297 premises.

*United States Department of Agriculture, Agricultural Research Service, Animal Health Division.
**United States Department of Agriculture, Agricultural Research Service, Biometrical Services.
†United States Department of Agriculture, Consumer and Marketing Service, Livestock Slaughter Inspection Division.
‡Iowa State University, College of Veterinary Medicine.
collected and processed. This paper reports the methods used in conducting this survey and the current incidence of *Trichinella spiralis* infection in garbage fed swine as revealed by the survey.

**LITERATURE REVIEW**

There have been numerous reports of the incidence of *T. spiralis* in garbage fed swine in the past. The most recent was a study initiated in April 1961 by the Meat Inspection Division, Consumer and Marketing Service, United States Department of Agriculture in cooperation with the Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University of Science and Technology. This study was completed in March 1965. Samples were collected from 5,041 garbage fed swine from most areas of the country. One hundred and thirty one (2.6 percent) were positive. The authors state that certain trends were noted during the survey, with the incidence decreasing sharply in certain areas from the early part of the survey to the latter part.

Kerr (1942) found that in California, of 1701 hogs fed raw garbage, 6.4 percent were infected; of 1109 hogs fed on grain and kitchen scraps, 0.6 percent were infected and that no hogs fed cooked garbage were infected.

Schwartz (1940) reported that between 1933 and 1937, of 6486 hogs fed uncooked garbage, 4.41 percent were infected and of 1987 fed cooked garbage 0.55 percent were infected.

From 1935 to 1957, inclusive, the Zoological Division of the Bureau of Animal Industry (now the Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture) in cooperation with the Meat Inspection Division examined the diaphragms of 55,848 hogs for trichinae by the digestion technique. These specimens were obtained from meat packing establishments throughout the country and were classified according to the ration the hogs were reportedly fed. Of these, 26,213 were reportedly fed garbage (8,837 fed cooked garbage and 17,376 fed raw garbage). The average incidence of trichinae in these garbage fed hogs was 5.65 percent.

**DESIGN AND CONDUCT OF THE SURVEY**

In designing a survey there are three items that are important to consider. The first is that the survey be designed so that the accuracy of the sample estimate may be determined from the sample itself providing an unbiased sample estimate. The second is that as much information as possible be obtained within the practical limits of the survey. The third is that the sample drawn be representative of the population being sampled.

To obtain an unbiased sample estimate with the accuracy being determined from the sample itself, it is necessary to use some form of random sampling. This means that each herd and each State in the population has a known chance (which is different from zero) of being in the sample. Tables of random numbers were used for this purpose.
The method used to obtain an accurate estimate while keeping the project within practical limits was to select only a portion of the States. The method of selecting only part of the States is known as cluster sampling with each State being considered a cluster of herds.

In order to provide a sample that was more representative of the population than would be one drawn by the use of simple random sampling (thus increasing the accuracy of the sample) three restrictions were placed upon the random sampling.

The first restriction was that the States of Massachusetts, New Jersey, and California were selected for the survey. This was done because these three States contain about 33 percent of the country's garbage fed swine. This does not destroy the requirement of randomness since these States have a probability of 100 percent of being in the survey. The only necessary requirement for randomness is that each State have some probability different from zero of being in the survey.

The second restriction was to divide the remaining 45 States of the continental United States into two groups. The groups were divided on the basis of the Animal Health Division's four regional areas. The Eastern group consists of the 23 States from the Northeast and Southeast regions. The Western group consists of the 22 States from the Midwestern and Western regions. Six States were selected from each group. This meant that each Eastern State had a probability of 26.1 percent of being chosen while each Western State had a probability of 27.3 percent of being chosen. The selected States are illustrated in Figure 1.

The third restriction was to rank the herds in each of the selected States from largest to smallest and then from each succeeding group of ten, select one herd at random. This type of sampling is known as stratified random sampling. This meant that no matter the size of the herds, an unselected herd was sure of being represented by a herd of similar size. Approximately 10 percent of the swine from each selected herd had samples taken at slaughter. Although 10 percent of the herds were taken from a State the stipulation was made that States having between 50 and 100 herds would have 10 herds selected while States having less than 50 herds would have five herds selected.

The above method of sampling is described as stratified cluster random sampling.3

The project in the field was coordinated by a representative* of the Animal Health Division's headquarters office who traveled to each participating State and assisted in initiating the survey.

Selected herds were visited by State or Federal regulatory personnel who explained the project to the herd owner. Herd owners were asked to notify the inspectors when swine were ready for slaughter. The inspector identified the swine, then notified the meat inspector at the particular slaughtering establishment where the swine were to be slaughtered. The meat inspector collected approximately three ounces of diaphragm from

*Mr. Harold K. Cline, United States Department of Agriculture, Agricultural Research Service, Animal Health Division, presently stationed at San Pedro, California.
each hog. Often times it was necessary for the field inspector to accompany the swine to slaughter for specimen collection when meat inspection was lacking.

Specimens were dusted with boric acid powder, placed in separate plastic bags, identified as to farm of origin, and shipped to the Veterinary Medical Research Institute, Iowa State University, where they were examined by the artificial digestion—Baermann technique.

When blood samples were collected in addition to the diaphragm specimens, both the blood and the diaphragm were identified with the same individual code number so results of one test could be keyed to the results of the other.

Generally ten percent of the animals in a herd going to slaughter were sampled. On occasion, it was discovered that selected herds were no longer in business or were raising pigs only to sell as feeders. In these instances, herd substitutions were made, again employing tables of random numbers.

To assist in evaluating the possibility of why one premises was infected and another was not, a short history of the husbandry practices of each premises sampled was completed. Data collected included (1) number
and type of swine, (2) raised or purchased, (3) farm sanitation, (4) rodent infestation, (5) wild animals on premises, (6) method of disposal of dead animals, (7) health of swine, (8) adequacy of cooking, (9) condition of equipment, and (10) are table scraps fed.

ANALYSIS OF RESULTS

The data was analyzed with the aid of methods described in Cochran and Hanson, et al. The results are shown in Table II while Table I shows the population involved in the various States along with the weighting percentages for the various States and regions.

TABLE I
Population Sizes, Representation and Weights for Calculating Trichinae Incidences

<table>
<thead>
<tr>
<th>State and Region</th>
<th>Population for Sampling</th>
<th>Percent Population Represented</th>
<th>Population for Weighting</th>
<th>Weighting Percent</th>
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</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>34,157</td>
<td>97.8</td>
<td>33,413</td>
<td>31.03</td>
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<td>Louisiana</td>
<td>19,186</td>
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</tr>
<tr>
<td>Selected</td>
<td><strong>830,473</strong></td>
<td><strong>98.3</strong></td>
<td><strong>830,473</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

*Figures at time herds were selected.
**Figures from May 1966 Report.
Because the sampling was done by splitting the United States into regions and breaking the States into strata by size of herd, the incidence estimates for the various States and the United States could not be calculated directly from the number of positive and total samples. Instead, the proportion of swine in each group of herds and in the various States had to be considered. For example, the last step in calculating the incidence for the United States was as follows:

<table>
<thead>
<tr>
<th>Percent of Swine</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>East</td>
<td>43.40</td>
</tr>
<tr>
<td>West</td>
<td>23.43</td>
</tr>
<tr>
<td>Deliberately Selected States</td>
<td>33.17</td>
</tr>
</tbody>
</table>

**TABLE II**

Incidence of *Trichinella spiralis* in Garbage Fed Swine
July 1964 – July 1966, by State and Region

<table>
<thead>
<tr>
<th>State and/or Region</th>
<th>Number of Samples</th>
<th>Number Animals Infected</th>
<th>Number Premises Sampled</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>539</td>
<td>0</td>
<td>77</td>
<td>0.0 %</td>
</tr>
<tr>
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<td>213</td>
<td>0</td>
<td>53</td>
<td>0.0</td>
</tr>
<tr>
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<td>174</td>
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<td>8</td>
<td>0.8306</td>
</tr>
<tr>
<td>Mississippi</td>
<td>41</td>
<td>1</td>
<td>9</td>
<td>0.9096</td>
</tr>
<tr>
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<td>330</td>
<td>4</td>
<td>16</td>
<td>1.5036</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>126</td>
<td>0</td>
<td>5</td>
<td>0.0</td>
</tr>
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<td>Arizona</td>
<td>84</td>
<td>2</td>
<td>10</td>
<td>2.0356</td>
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<td>Colorado</td>
<td>723</td>
<td>1</td>
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<td>0.1719</td>
</tr>
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<td>3</td>
<td>0.0</td>
</tr>
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<td>9</td>
<td>1.3320</td>
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<td>0.0</td>
</tr>
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<td>Oregon</td>
<td>104</td>
<td>0</td>
<td>5</td>
<td>0.0</td>
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<td>746</td>
<td>0</td>
<td>17</td>
<td>0.0</td>
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<tr>
<td>Massachusetts</td>
<td>1038</td>
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<td>31</td>
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<td>168</td>
<td>0.5372</td>
</tr>
<tr>
<td>Western</td>
<td>1369</td>
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<td>62</td>
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<td>Deliberately Selected States</td>
<td>3163</td>
<td>13</td>
<td>67</td>
<td>0.3772</td>
</tr>
<tr>
<td>United States</td>
<td>5955</td>
<td>25</td>
<td>297</td>
<td>0.50014</td>
</tr>
</tbody>
</table>
The percent of swine is shown in Table I while the incidence for United States = \((0.4340 \times 0.005372) + (0.2343 \times 0.006055) + (0.2217 \times 0.003772)\) = 0.0050014 or 0.500 percent.

The incidence estimates for the various States were calculated by applying similar weights to the various groups of herds. In New York for example, there was one infected herd. The incidence in this herd was four infected out of the 32 animals samples giving a herd incidence rate of 0.125, while the group of herds represented by this herd had 12 percent of the garbage fed swine in New York. Consequently, the New York State incidence is as follows: Incidence = 0.125 \times 0.120 = 0.015 or 1.5 percent.

In a few instances it was not possible to sample all of the selected herds in the States. For this reason the swine represented by the sampled herds were used to figure the weight for each State in calculating the incidence for the regions and the Nation rather than the total swine in each State. Nationwide, 98 percent of the swine were represented and representation for the various States is shown in Table I.

The incidence estimates for the various States, regions, and the United States are shown in Table I. The national incidence is 0.0050014 (0.500 percent.) In order to determine the accuracy of this estimate, the standard error was calculated. The standard error is 0.00130. A formula from Cochran\(^2\) was used to calculate the standard error. The 95 percent confidence limits of the estimate is approximately two standard errors\(^3\) or 0.00260. This means that the probability is 95 percent that the true incidence is 0.50 percent ± 0.260 percent or between 0.240 percent and 0.760 percent.

The distribution of larvae per gram is shown in Table III along with the distribution determined by the Meat Inspection Division survey conducted April 1961 to March 1965.\(^8\)

It is interesting to note that the two distributions are the same indicating that the degree of infection in infected swine is similar even though the incidence of infection was much lower on the present survey.

**TABLE III**

<table>
<thead>
<tr>
<th>Trichinae per gram</th>
<th>Total</th>
</tr>
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<tr>
<td></td>
<td>&lt;1</td>
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<tr>
<td>Meat Inspection Survey</td>
<td>79 (60%)</td>
</tr>
<tr>
<td>This survey</td>
<td>15 (60%)</td>
</tr>
</tbody>
</table>

**DISCUSSION AND CONCLUSIONS**

A project of this magnitude cannot be successfully conducted without the cooperation and work of many individuals. It is conservatively estimated that 600 individuals, including swine owners, State and Federal
livestock inspectors and veterinarians, meat inspectors, and meat packing industry personnel in 15 States played some role in the survey. In several instances personnel in States not engaged in the survey were called upon for help when swine from selected premises were being slaughtered in other than their home State. The enthusiasm displayed by almost all those participating in all aspects of the survey must certainly be taken as an indication that there is high interest in trichinosis in the United States today.

Unfortunately the brief herd history that was completed for each premises surveyed did not reveal any striking management practices common to infected premises, nor did the histories indicate significant differences between infected or noninfected premises. In some instances there were indications that cooking and husbandry practices were not always up to par; however, this occurred no more often on infected premises than on noninfected premises.

Although it was not intended that epidemiological investigations to determine most probable sources of infection be conducted during the survey, it was hoped that, through the short herd histories, more definitive information as to possible sources would be learned. There is a need for more work in this area.

In addition to diaphragms, blood samples, individually identified to their respective diaphragms were collected from over 5,000 animals. Sera from these samples are now stored at the Animal Disease and Parasite Research Division's Parasitological Laboratory at Beltsville. A very important need is the development of a diagnostic test to detect trichinae in the living animal. If the development of a serological test for this purpose is undertaken, having available a large number of sera from known infected and noninfected animals in the field would be invaluable.

The above data indicates that the level of trichinae infection in United States garbage fed swine is presently the lowest ever reported. It seems probable that two factors may be responsible for the low incidence revealed by this survey: (1) an actual decline in incidence brought about by the reemphasis of garbage cooking requirements in all States because of the hog cholera eradication program, and (2) the statistical design of the survey with rigid adherence to the protocol throughout which prevented the continuous sampling of animals which were more likely to be infected. It is readily ascertained that the trichinosis problem is not confined to the garbage fed swine when incidence figures are converted to the actual number of animals infected. Using swine population figures reported by the United States Department of Agriculture the number of infected garbage fed swine in the continental United States at any one time would be approximately 4,200. On the other hand the number of infected grain fed swine (recent studies show an incidence of 0.12 percent in farm-raised, butcher-weight swine and 0.22 percent in farm-raised breeder swine) at any one time would approximate 50,000. It is obvious, then, that any program contemplated to eradicate trichinosis, must be aimed at both the garbage fed hog and the grain fed hog.
REFERENCES

INTERFERENCE BY TRANSMISSIBLE GASTROENTERITIS IN THE IMMUNIZATION OF PIGS AGAINST HOG CHOLERA WITH CRYSTAL VIOLET GLYCEROL VACCINE

J. P. Torrey, B.S., D.V.M., M.S.

INTRODUCTION

A study of records kept for five years on farm swine herds vaccinated each year with crystal violet glycerol (CVG) hog cholera (HC) vaccine revealed that some herds were not protected against hog cholera virus (HCV) by a single dose of vaccine. Four of these herds that were known to be infected with Pasteurella spp. were adequately protected when a second dose of CVG vaccine was given.\(^2\)

This paper reports the results of a study of a farm swine herd composed of three lots of different age pigs that were naturally infected with transmissible gastroenteritis (TGE) at the time when they were vaccinated against HC.

MATERIALS AND METHODS

**Virus.**—The HCV used in this experiment for virulent virus challenge was prepared from lyophilized virus blood that had been kept at about 40 F. for 17 years. The reconstituted virus blood was injected into a HC susceptible pig that was exsanguinated the seventh day after injection. The blood was defibrinated mechanically, filtered through sterile cotton to remove the fibrin, identified as virus s.n. 318, and stored in 100-ml. bottles at -70 F. The virus was free from variant characteristics and was fatal to pigs in a dilution of 10\(^{-6}\).

**Vaccine.**—The method of preparation and testing CVG vaccine used in this experiment has been described.\(^1\) The vaccine used in this experiment was made of six lots totaling 128 liters and designated as s.n. 116. One ml. doses protected 60-lb. experimental pigs against two ml. of virulent HCV. The percentage of protection when determined by a previously described method\(^2\) was 89.3. It was also used on farm herds totaling 10,066 head with 71.6 percent protection.

**Herd History.**—The pigs raised each year on this farm had been vaccinated with CVG vaccine for five years before the time of this report. The vaccination records for the five years show vaccination of 2,099 pigs from 11 different farrowings with four to six vaccinated pigs selected for challenge inoculation from each farrowing. A total of 50 pigs given challenge inoculations for immunity protection showed an average protection of 70.6 percent for the five-year period. This is practically the same

From the National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

358
immunization against HC with CVG vaccine

359

protection for all other farm herds vaccinated in the five-year period.

Some pigs were selected each year to replace the older sows and the sows that were not productive. Some sows were kept as long as three years. Two litters of pigs were raised each year from each sow. No indication of disease in the sows or pigs had been observed during the five-year period.

The sows that produced the pigs involved in this report were in good health and had been bred so they would farrow in three groups about four weeks apart. The first group of 30 sows farrowed two weeks prior to the time when they and the pigs became affected with TGE. A clinical diagnosis of TGE was made by a practicing veterinarian and the diagnosis was confirmed by the State Diagnostic Laboratory. The pigs and sows were treated with antibiotics and given special care. An average of three pigs per litter survived and no sows died. The remaining pregnant sows were moved to another area, but they soon had signs of the disease that spread slowly through the herd. The second group of sows was infected before they began to farrow and still had some signs of TGE at farrowing time. The pigs from these sows also had symptoms of a mild form of scours, but none of the pigs died. The third group of sows to farrow had recovered from the TGE infection before the pigs were farrowed, and no signs of infection were observed in the pigs.

Pigs.—Each age group of pigs was kept separate until weaning time when they were moved into the same pasture and fed from self feeders containing a balanced ration. When the groups of pigs were 10, six, and two weeks of age, all three groups of pigs were vaccinated against HC with CVG vaccine. Each pig was injected with five ml. of vaccine subcutaneously posterior to the point of the elbow. All the pigs appeared to be normal except a few six-week-old pigs that had some signs of scours. One month later, 20 pigs from each age group were given a second five-ml. dose of CVG vaccine and identified by ear markings. All the pigs were kept in the same pasture until the older pigs were four months of age, then all were moved to a concrete feedlot. After they had been in the feedlot 30 days, five single-vaccinated and 10 double-vaccinated pigs were selected and tagged from each of the age groups. The selected pigs remained in the feedlot until five months after the first vaccination. When they were moved to the National Animal Disease Laboratory, single- and double-vaccinated pigs of each age group were placed in separate isolation stalls and each pig was injected subcutaneously with two ml. of virulent HCV s.n. 318. Only two of the youngest single-vaccinated group were obtained. The other three had been sold by mistake.

Daily observations were made and the condition of each pig was recorded by a point system that was used to determine the percentage of protection. The absence of sickness or the smaller number of days each pig was sick after challenge inoculation with virulent HCV was considered as indicative of the rate of recovery from virus exposure. On days zero, three, four, five, six, seven, 11, 13, and 14 after challenge inoculation, the temperature of all pigs were taken, and on days three, five, seven, 10, 12, 14, and 17 after challenge inoculation, white blood cell (WBC) counts were
<table>
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<th>Pig No.</th>
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<th>Days after challenge inoculation temperatures were taken</th>
<th>Vaccination Days after challenge inoculation temperatures were taken</th>
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<td></td>
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<td>0</td>
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<tr>
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</tr>
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Average for Single: 102.8, 105.8, 106.2, 105.0, 104.4, 104.6

Average for Double: 102.6, 105.9, 106.7, 105.2, 105.6, 104.6
<table>
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<th>Vaccination</th>
<th>Days after challenge white blood cell counts were made</th>
<th>Avg. Percent Protection</th>
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<td>Days No.</td>
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</tr>
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<td>7650</td>
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<td>11200</td>
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made. Feed was withheld 24 hours before blood was drawn for WBC counts. All surviving pigs were observed daily for 40 days after injection and considered normal. Gross necropsy examinations for HC lesions were made on the pigs that died and pieces of the heart, lungs, liver, spleen, and kidney were taken aseptically and cultured for bacteria on blood agar.

RESULTS

After CVG vaccination, there was no visible reaction from the vaccine or any indication of TGE infection in the herd. The pigs made normal gains and all appeared to be in good health. The oldest pigs averaged about 300 lbs. at time of challenge and the youngest group averaged about 250 lbs. at this time. The middle age group of pigs was uneven in size, some weighing approximately 300 pounds and others about 250 pounds.

The daily temperatures of each pig and the average daily temperatures of each group of pigs were given in Table I. The temperatures of all pigs before injection were normal (104.0 F. or less).

On the third day after injection of HC challenge virus, all of the single-vaccinated pigs and all except four of the double-vaccinated pigs had a rise in temperature. Some temperatures were as high as 107.0 F. There were two pigs that had no rise in temperature throughout the test period. The temperatures of the pigs that survived reached the peak on the third or fourth day, and most of the temperatures had returned to normal by the seventh day. Temperatures of seven pigs exceeded 107.0 degrees and five of these died. The average temperatures for the single-vaccinated pigs were higher than the average temperatures of the double-vaccinated pigs in each age group.

The average WBC count of single- and double-vaccinated pigs for each age group of pigs is shown in Table II. On day 0, before HCV was injected, the average WBC count of the single-vaccinated pigs was 21,795 and of the double-vaccinated pigs was 21,354. The average WBC count of all groups of pigs dropped rapidly by the third day after injection of virus. There was only one pig in each of the three single-vaccinated groups with a WBC count of more than 10,000 cells per cmm. The WBC counts of the other pigs in these groups were below 10,000. In the double-vaccinated pigs, the WBC counts did not drop below 10,000 in two pigs vaccinated at two weeks of age, in five pigs vaccinated at six weeks of age and in five pigs vaccinated at 10 weeks of age. The counts of the other pigs in the double-vaccinated groups were slightly under 10,000 on the third day after injection of virus. The average WBC counts of all the groups of pigs began to increase after the third day.

The average WBC counts of the double-vaccinated pigs in each age group increased more rapidly, reached a higher level, and returned to normal more rapidly than the WBC counts of the single-vaccinated pigs of the corresponding age group (Table II and Graph 1). There was not much difference in the maximum average WBC count of the single- and double-vaccinated pigs given the first injection of vaccine at two weeks of age.
IMMUNIZATION AGAINST HC WITH CVG VACCINE

Graph 1. Average white blood cell counts of pigs single- and double-vaccinated at different ages with crystal violet glycerol vaccine and exposed to virulent hog cholera virus five months later.

The average WBC count of the pigs that received a single dose of vaccine at six weeks of age increased more slowly and reached a lower level (27,000) than the other groups of pigs that received single doses of vaccine. The average WBC count of the pigs vaccinated at six weeks of age and given a second dose of vaccine one month later increased more rapidly and reached a higher level (36,000) than the other double-vaccinated groups. This was the group of pigs that was affected with TGE virus at time of vaccination.

The number of days the pigs were sick after challenge with virulent HCV was fairly constant for the survivors of all groups of pigs. There was about one day difference in the number of days of sickness between the single-vaccinated and double-vaccinated survivor pigs in each group.

The percentage of protection for the pigs given one dose of vaccine at two, six, and 10 weeks of age was 43.0, 14.8, and 58.8, respectively.
Protection of the single-vaccinated pigs was less in each age group than in the double-vaccinated pigs with percentages being 70.4, 74.6, and 79.8, respectively. The percentage of protection of the six-week-old, single-vaccinated pigs was low and four of the five pigs died. The percentage of protection of the double-vaccinated pigs increased with the age of the pigs at time of vaccination. This was also true of the pigs given one dose of vaccine at two and 10 weeks of age.

Lesions typical of HC were observed in all the pigs that died and no pathogenic organisms were isolated from tissues examined.

**DISCUSSION**

Veterinarians who vaccinate pigs against hog cholera know that a herd should not be vaccinated if the pigs are sick or do not appear to be healthy. However, it is not always possible to detect when a herd is in the incubative stage of an infection or when there is some latent infection present. The herd of pigs discussed in this report appeared normal except for scouring of a few pigs in the six-week-old group, and this might have been attributed to something other than TGE. Since this herd had no known infection for a period of five years and no pigs had been brought into the herd and TGE had been diagnosed in the herd, it seems logical to attribute the low immune response to TGE.

Haelterman stated that apparently the resistance demonstrated by pigs against TGE was not dependent upon circulating antibody. He stated, however, that pigs that received either milk from immune sows or those fed antiserum were resistant, and that this resistance was dependent upon the continuing presence of immune milk or antiserum in the alimentary tract. He concluded that protection transmitted to pigs by immune sows was not conferred by the absorption of globulin from colostrum, but depended on a continuous supply of antibody in the milk from the dam. These conditions may account for the results obtained with the six-week-old pigs in this report.

The second lot of sows in this report had probably not recovered sufficiently from TGE infection to produce significant antibody in their milk. The antibody was sufficient to keep the pigs from dying but not to prevent scouring. The third group of sows had recovered from the infection before the pigs were farrowed and apparently produced milk with enough antibody to prevent the pigs from becoming sick even though they were exposed to TGE. The first group of pigs had the disease and were actually immune survivors.

A great deal of work has been done on interference by living viruses and also by inactivated viruses but little has been done to show this interference of a live virus on the action of a killed virus. It seems logical to suppose that the action of a live virus would be greater against a killed virus. Much work has also been done on interference clearly establishing it as an intracellular phenomenon. The dosage and timing of the two viral agents determine to a large degree what the end results may be. This may explain why the middle age group of pigs in which the TGE
infection was most active responded so poorly to a single dose of vaccine and why the other two groups' responses were better. It is very evident that the immune responses of all three groups of pigs were inadequate when the percentage of protection (43.0, 14.8 and 58.8) of the two, six, and 10-week-old pigs was compared with the 71.6 percent protection for the vaccine on farm herds and 89.3 percent protection on experimental pigs.

The mechanism of interference is not clearly understood. But, since it is a cellular phenomenon, then those cells that would have normally responded to a single dose of CVG vaccine in producing immunity were in some way prevented from doing so, or at least to a lesser degree. The question arises as to what effect a single dose of vaccine had on the cells so that a second dose of vaccine produced adequate immunity. This difference in the results of the two vaccinations is clearly demonstrated when the percentage of protection of a single dose of vaccine on the two, six, and 10-week-old pigs (43.0, 14.8, and 58.8, respectively) is compared with the 70.4, 74.6 and 79.8 percent protection of the double dose on the comparable pigs.

The reactions to single- and double-vaccination are reflected in the WBC count of the pigs after challenge with virulent HCV. The WBC count of the middle age, single-vaccinated pigs increased more slowly and reached a lower level than any other group of pigs. The WBC count of the double-vaccinated pigs in this group increased more rapidly and reached a higher level than any other group of pigs (Table II and Graph 1).

The degree of reaction as reflected in the temperature and WBC count indicates that pigs vaccinated with two doses of CVG vaccine do not have as severe a reaction to HC as pigs given one dose of vaccine. If less than 10,000 WBC count is considered leukopenia, then the double-vaccinated pigs are less likely to develop leukopenia than single-vaccinated pigs. The more rapid increase in WBC count of double-vaccinated pigs indicated more prompt recovery.

Results similar to those obtained after single- and double-vaccination of this herd affected with TGE have been obtained in herds infected with Pasteurella.²

SUMMARY

When pigs that were naturally infected with transmissible gastroenteritis (TGE) were vaccinated against hog cholera (HC) with a single dose of crystal violet glycerol (CVG) vaccine, the TGE infection interfered with protection against HC. When the pigs were given challenge inoculations with virulent hog cholera virus (HCV), they developed a marked leukopenia and four of five pigs died. When the pigs were given a second dose of CVG vaccine one month after the first dose, their average white blood cell count did not reach a leukopenic stage and it returned to normal more rapidly than in pigs vaccinated one time; one of 10 pigs died.

Pigs immune from an active infection of TGE or protected against TGE by drinking colostrum milk from immune sows were protected
against HC by a single dose of CVG vaccine so that only one pig died in each group when challenged with virulent HCV. A second dose of CVG vaccine increased this protection greatly. No deaths occurred in these two groups when two doses of CVG were given.

Two doses of CVG vaccine administered one month apart produced better protection in all groups of pigs than a single dose of vaccine.

REFERENCES


REPORT OF THE COMMITTEE ON TRANSMISSIBLE
DISEASES OF SWINE


In general the problems considered have been much the same as those of the past year. The emphasis has shifted in some instances. Conflicting views presented seem to have been those differences which largely characterize objectivity vs. expediency. While the spectrum of attention is wide, the membership has given considered attention to details of problems of current and continuing importance. The following items are recommended to the Association for adoption as the report of this Committee.

1. Jowl Abscesses

Abscesses of the pharyngeal and cervical lymph nodes appear to be caused by group E. Streptococci although it is recognized that other microorganisms have been isolated from such abscesses. Reports have indicated that the incidence is rising. In 1948 condemnations in inspected swine were 1.6 percent and rose to 3.4 percent in 1964. Condemnation of one percent of carcass parts resulted from all other causes. The estimated annual loss at Federally inspected slaughtering houses exceeds $12,000,000.

It is recommended that research be encouraged and expanded on this costly disease. Studies which will lead to means of prevention and control of jowl abscesses should be undertaken without delay. The recent appropriation from the Congress of the United States is commended as the initiation of public support for research on this disease. Attainment of program goals can only be assured through sustained effort.

2. Trichinosis

The public health aspects of trichinosis infection among swine is of importance in the acceptance of pork by the housewife. Recent surveys by State and Federal workers reveal that, coincident with vigorous enforcement of garbage cooking laws, the incidence of the disease has declined among garbage fed swine. There is also a low incidence among grain-fed swine. In spite of the low incidence, adverse publicity associated with trichinae infections has had a negative impact on retail sales of fresh pork products.

Further studies are needed to—
1. Detect the presence of trichinae in living animals;
2. Determine sources of infection.

3. Sale of Boars from Testing Station for Breeding Purposes

Concern with the inherent danger of returning breeding animals to farms from concentration points such as boar testing stations is reaffirmed. Inapparent diseases acquired under such circumstances such as, atrophic rhinitis, virus pneumonia, hog cholera, pseudo-rabies, mycoplasmosis, salmonellosis and enterovirus infection are examples of diseases which could go unrecognized to the farm of the purchaser, subsequently cause considerable loss, and not be readily referable to the actual source. The feasibility of "on the farm" boar testing should be further investigated as a possible alternative.

4. Enforcement of Garbage Cooking for Swine

The garbage laws of all states should continue to be vigorously enforced. There are between eight and ten thousand feeders of garbage to swine, feeding between 700 and 900 thousand swine during each month and about one percent are being fed raw garbage. It is difficult to clearly evaluate the obvious threat of diseases which may be transmitted by such practice, and the further threat to human health as the pork product is consumed. It is easier to prevent the spread of disease by this means than to define the measure of damage if the regulations are lightly held.

5. Transmissible Gastroenteritis of Swine

The report of the Subcommittee on Establishing Criteria for the Diagnosis of Transmissible Gastroenteritis (TGE) of swine is accepted. The current perspective of the problem is well presented. It is recognized that research efforts will probably resolve the controversy surrounding the nature of the etiologic agent. The report of the Subcommittee is included as an addendum to the report of the Committee. The Subcommittee has been requested to continue to evaluate progress that is being made in the study of TGE.

6. Proposed Avian Type Tuberculosis Eradication Program

A proposal for the systematic eradication of Avian type tuberculosis was received from the Animal Health Division of the United States Department of Agriculture. The proposed program is approved in principle and is commended to the Committee on Tuberculosis and Paratuberculosis for more detailed study and further support.
TRANSMISSIBLE DISEASES OF SWINE

REPORT OF THE SUB-COMMITTEE* ON ESTABLISHING CRITERIA FOR THE DIAGNOSIS OF TRANSMISSIBLE GASTROENTERITIS OF SWINE


Transmissible gastroenteritis (TGE) is usually recognized as a highly fatal enteric disease of piglets under two weeks of age. However, swine of all ages are susceptible to this viral infection, but shoats and older animals may show no clinical signs or only a transient diarrhea.

The accurate diagnosis of TGE is needed for a better understanding of: (1) its economic importance; (2) its epidemiological character, including its incidence, prevalence, and mode of transmission; and (3) its method of control, including the use of vaccines. Recently, much research has been devoted to TGE. Some of the findings are of value in diagnosing this disease.

The purpose of this report is to review and attempt to evaluate the present methods of diagnosing this disease. It is realized that insufficient data is available for a proper evaluation of the newer laboratory methods.

Clinical Signs and History

The typical clinical signs of TGE in piglets consist in: (1) sudden transient vomiting, (2) accompanied or rapidly followed by a watery and usually yellowish diarrhea, (3) dehydration, and (4) high morbidity and mortality in pigs under two weeks of age. Signs in shoats and sows may be limited to diarrhea for one or a few days. Some lactating sows become very sick, with an elevated temperature, agalactia, vomiting, inappetance, and diarrhea. An accurate diagnosis of TGE can usually be made if piglets and some of the shoats or sows in the same herd show the clinical signs as described. In a herd containing only shoats or older swine, an explosive outbreak of diarrhea would be suggestive of TGE, but laboratory methods would have to be used for an accurate diagnosis.

Subclinical infection is rather common in non-lactating sows, less so in shoats. A moderation in the clinical signs probably occurs when partially immune swine are exposed, but reliable information on this point is lacking.

Other disease which produce severe diarrhea in piglets include: (1) Colibacillosis. However, the following clinical features are usually much more pronounced with TGE: vomiting, rapid spread from one litter to another, acute course, morbidity, and mortality in pigs under two weeks. TGE appears as a severe epidemic usually terminating abruptly, whereas colibacillosis may be a persistent herd problem. Invariably, in

*This is a Sub-committee of the Committee on Transmissible Diseases of Swine. Three members are from this Committee and three from the Conference of Veterinary Laboratory Diagnosticians.
TGE, some sows and shoats in the herd will have a severe diarrhea, but such animals are not so affected in colibacillosis. (2) Enterotoxemia due to *Clostridium perfringens* type C. This condition is characterized by blood in the stool and by hemorrhage in some portions of the small intestine. It is most often seen in pigs under three weeks of age. The hemorrhagic nature of the diarrhea and enteritis should serve to distinguish it from TGE. Undoubtedly, there are other diseases of piglets which exhibit diarrhea and which might simulate TGE but such have not been adequately described to date.

TGE occurs primarily in the cold months, probably due to the greater stability of the virus at lower temperatures outside of the host's body. This, in turn, facilitates its spread from one herd to another.

*Post-Mortem Lesions*

*Gross lesions*—Besides dehydration, gross lesions are usually confined to the gastrointestinal tract. The stomach is often distended with curdled milk, especially if the pig dies in the early stage of the disease. Congestion of the gastric mucosa will be seen in some, but not all, pigs.

The small intestine is distended with yellow and frequently foamy fluid. The wall is thin and almost transparent. The ingesta is incompletely digested. The gross lesions are not usually considered to be pathognomonic for TGE, as similar lesions can be seen in colibacillosis.

*Sub-gross lesions*—Atrophy of the intestinal villi has been described by Haelterman and Hooper as a consistent finding in TGE, and this observation has been confirmed by several other workers. This atrophy is most pronounced in the jejunum and ileum but is variable in the duodenum. Examination of the villi may be made by opening short segments (about one inch in length) of the jejunum, placing in a petri dish containing water, and viewing the exposed mucosa at a low magnification, using a dissecting scope or a strong hand lens. How specific villous atrophy is for TGE is not known, although preliminary observations indicate that it is not seen in colibacillosis.

*Microscopic lesions*—The only microscopic lesions that are known to be of diagnostic significance are those that are related to villous atrophy.

*Clinical Pathology*

At present, there have been no reports of significant alterations in blood counts, or in blood and urine analyses that could be utilized for diagnostic purposes.

*Serologic Tests*

A diagnosis can be made by detecting TGE antibodies in recovered animals, preferably using both acute and convalescent serum samples. Three methods of detecting TGE antibodies have been described: (1) A neutralization test, using pigs as the indicator system. This method is not practical for routine diagnostic purposes because of the cost of susceptible pigs. (2) A neutralization test, using cell cultures (usually from porcine kidneys) as the indicator system. This method has been used in
several laboratories and the results indicate it to be reliable and of great value in confirming a clinical diagnosis. A cytopathic strain of TGE virus is necessary for conducting this test. Preferably, serum samples taken from sows in the acute and convalescent (two weeks later) stages of the disease are tested. The former animals should be negative, while the latter will usually have titers of 1:64 or above. Two methods are available for determining the extent of virus neutralization: inhibition of CPE and plaque reduction. Both methods are satisfactory. The latter gives a more distinct end point but may be more complicated to conduct.

A bentonite agglutination (BA) test has been described recently. In this test, TGE antibodies will agglutinate bentonite particles on which TGE virus has been previously adsorbed. According to Ristic, the most accurate diagnoses using this test can be made if paired serum samples are secured; the first sample taken at the time of the sickness of the litter and the second sample taken four to five weeks later. As yet, this test has not been as extensively investigated as the neutralization test.

Detection of TGE Virus

The highest concentration of virus occurs in the small intestine during the first few days following infection. Thus, a segment of the small intestine, collected early in the disease, is the preferred specimen for detecting TGE virus. Several methods are available:

1. Inoculation of susceptible piglets. The gut or fecal suspension (see appendix for preparation) is orally administered to susceptible piglets, preferably only a few days old. Vomiting and a severe diarrhea, starting in 18 hours to three days, is usually considered satisfactory proof for the presence of TGE virus.

2. Cell culture methods. Primary porcine kidney cultures prepared from young or fetal pigs have been used most commonly. Such cultures are inoculated with a suitably prepared gut or fecal suspension (see appendix). The cytopathic effect (CPE) by field strains is usually transient or negligible in early passages, and its detection will depend on the susceptibility of the cell culture and the experience of the observer. More distinct CPE will be observed after five to 10 cell culture passages made at about six-day intervals. The plaque technique has been reported to be more sensitive and satisfactory for primary isolation. Plaques will often occur on primary isolation, and they are much more easily recognizable than the transient, slight CPE. Identification of the CPE or plaque-producing agent as TGE virus is accomplished by neutralization with specific anti-TGE serum. However, it should be stated that some investigators believe that this CPE virus is not the true cause of TGE, but, instead, give the credit to another virus which, it is claimed, replicates in cell cultures but does not produce a CPE. Very little information is available, as yet, on the characteristics of this latter, non-cytopathic virus. Further research may be necessary to clarify this apparent discrepancy on the etiology of TGE.

3. Fluorescent antibody test. Research is in progress in several laboratories to determine if this test is applicable for the diagnosis of
TGE, and, if so, it should be of considerable value. In the test, smears or sections of the small intestine would be stained with a fluorescent-tagged TGE antibody. The TGE virus antigen would then be observed, intracellularly, as a fluorescent mass, as viewed by ultraviolet microscopy. The test may also be used to determine if cell cultures have been infected with TGE virus, since CPE is not readily evident in the early cell-culture-passages of field viruses.

(4) Bentonite agglutination inhibition (BAI) test. This test has been reported recently, but in its present form is rather complicated and probably not applicable for diagnostic laboratories. According to Ristic, "this test appears more suitable for the detection and titration of the virus in tissue cultures rather than in the gut contents of infected baby pigs because when the latter material is used, several purification steps are required before it can be used in the test."

SUMMARY

A diagnosis of TGE in piglets can usually be made on the basis of the clinical signs and history. For example, if some of the shoats or sows in the herd show severe diarrhea along with the typical clinical signs (vomiting, watery diarrhea, etc.) in piglets, a diagnosis of TGE is usually justified. In a herd containing only shoats or older swine, an explosive outbreak of diarrhea would be suggestive of TGE. In partially immune herds a deviation from the classical clinical signs can be expected. In these situations, laboratory methods must be used to obtain a correct diagnosis.

Laboratory methods that assist in, or confirm, the diagnosis of TGE are the detection of: (1) villous atrophy in the small intestine, (2) TGE antibodies, and (3) TGE virus.

A marked shortening of the villi in the jejunum and ileum represents a constant finding. At present, this lesion has not been associated with any other diarrheal disease in piglets.

With the availability of a TGE virus that is cytopathic for cell cultures, neutralizing antibodies can be detected in the serum of convalescent animals. This represents a very reliable method, and is probably the laboratory method most applicable for the diagnosis of questionable cases of TGE, especially if acute and convalescent serum samples are used. The bentonite agglutination test may prove equally valuable for detecting antibodies although it has not been as extensively investigated.

The examination of gut or fecal specimens for TGE virus can be accomplished by the inoculation of either piglets or cell cultures. The use of piglets is rather expensive but reliable, while the use of cell cultures, at present, is more of a research than a diagnostic tool. The fluorescent antibody technique for detecting TGE virus antigen in the intestinal cells of infected pigs or in inoculated cell cultures may prove to be of considerable diagnostic assistance in the future.
APPENDIX

PREPARATION OF FECAL AND GUT SUSPENSIONS

1. Homogenize in a small amount of physiological saline (or, other suitable diluent) using a mortar-pestle and sand or Waring blender or Virtis homogenizer. Usually, approximately a 10 percent gut or fecal suspension is made.

2. Centrifuge at approximately 3000 x g for 30 minutes, preferably in a refrigerated centrifuge. Carefully remove and save the supernatant fluid.

3. The supernatant fluid should be treated so as to remove or inactivate bacteria and fungi. Usually, either of the two following methods are satisfactory, with the latter preferred.
   (a) Add penicillin, streptomycin, and mycostatin at levels of 1000 i.u., 1000 micrograms, and 500 i.u. per ml., respectively.
   (b) Filter through a bacteriological filter, such as a 450 millimicron, Millipore filter.

REFERENCES

THE ROLE OF MEAT INSPECTION IN PUBLIC HEALTH

Jerome Payton, D.V.M.*

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In the short period of allotted time, it would be presumptuous or im-pertinent of one to believe he could do more than gloss over the very high-lights of the more pertinent material relating to the public health aspects of meat inspection. This paper cannot be exhaustive or detailed but attempts to bring forth some degree of the more relevant and current information.

Prior to the advent of the bacteriological period, attempts made in regulating food processing and slaughtering establishments have been di-rected mainly to correction of public nuisances—odors, sewage and offal discharges. Attempts were made to prevent obviously spoiled foods from being consumed by the public. All this in accordance with the commonly held beliefs that visible dirt and filth were causes of illness; insects and worms arose from spontaneous generation. Rational, scientific efforts in meat hygiene were brought into play to protect the people upon the dis-covery of microorganisms and their relationship to disease in man and livestock. Veterinarians, particularly in Europe, assumed a major role in pioneering food hygiene programs. Our American program dates from 1890 when some degree of control of meat hygiene and inspection pro-cedures were applied to those items destined for export to certain Euro-pean countries. Dr. D. E. Salmon of the United States Bureau of Animal Industry was an early leader in attempting to effect higher meat inspec-tion standards. Not until 1906, largely because of Upton Sinclair's "The Jungle" did our modern meat inspection service come into being.

The principles of food hygiene are designed, of course, to prevent illness or disease that might be caused by food products. Foods of animal origin must first come from healthy, physiologically normal animals. These animals must then be slaughtered and resulting carcasses and meat products processed under sanitary conditions to preclude contamination and deterioration. It will not suffice to say that meat inspection can ade-quately protect the consumer if conducted on a partial basis. Only an animal-by-animal or bird-by-bird inspection conducted by competent per-sonnel prior to slaughter, during slaughter and in every step of processing procedures can eliminate all diseased animals or unwholesome parts from food supply channels.

The Meat Hygienist searches out and eliminates from food channels diseased and otherwise unfit carcasses; sees that meat and meat products are kept clean during all stages of preparation into articles of food, guards against adulteration, residues, harmful preservatives and other deleterious substances. He causes sound and wholesome meat to be branded as inspected and passed and prevents the use of false or deceptive labels and statements on meat foods. In addition to the above, the meat

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inspector supervises the destruction for food purposes all diseased, un-
sound, or otherwise unwholesome meat, meat by-products and meat food
products. Of no little importance is the maintenance of adequate safe-
guards against the possibilities of inedible products of animal origin find-
ing its way into food channels of trade either as is or as an adulterant.
Doctor Jennings states: "One of the salient features of a modern meat
inspection law and program is the control of inedible and condemned
meats and meat products. It is necessary to prevent such products from
gaining entrance, either by accident or by design, into the channels of food
and to prevent dissemination of diseases among animals and to man."

CLASSIFICATION OF MEAT-BORNE DISEASES

Meat-borne diseases have been basically classified by Doctor Dol-
man as:

1. MEAT-BORNE DISEASES OF CHEMICAL OR TOXICOLOGICAL ORIGIN.
2. ENDOGENOUS (INTRAVITAL) ANIMAL INFECTIONS TRANSMISSIBLE TO MAN BY MEAT (ZOOSES).
3. INFECTIONS AND INTOXICATIONS DUE TO EXOGENOUS (HUMAN AND ENVIRONMENTAL) CONTAMINATION OF MEAT AND MEAT PRODUCTS (BACTERIAL FOOD POISONING).

This classification may not be adequate and consistent in all respects
but it does provide a basis for orderly, reasonable grouping and discus-
sion.

MEAT-BORNE DISEASES OF CHEMICAL OR TOXICOLOGICAL ORIGIN

Fortunately, it is unlikely that the meat of any animal or bird com-
monly consumed by man is intrinsically poisonous. Even the flesh of ani-
mals dead as the result of eating such plants as ergotized grain and Senecio is unlikely to contain enough of the alkaloids to cause harm to the con-
sumer. Milk from animals grazing on white snakeroot or rayless golden-
rod may convey termatol poisoning (the "MILK SICKNESS" of frontier
days). It is thought Abraham Lincoln's mother died of "MILK SICKNESS" in Illinois in 1818. While such alkaloids and other toxic substances seldom accumulate in high concentration in muscle tissue, this may not be true for viscera. Visceral organs may be intrinsically dangerous in other ways. Eskimos and explorers are well aware of the Vitamin A toxicity of the liver of the artic fox and the polar bear.

TOXIC CHEMICALS

Livestock naturally ingesting plant life containing appreciable
amounts of selenium or fluorine rarely contain sufficient amounts of these elements in their muscle tissues to be injurious if consumed. Toxic
substances have been added to meats and meat food products in the past primarily as preservatives. These substances were salicylic, boric, benzoic acids or their salts and formalin. These have long been banned. In the United States, sodium benzoate in a maximum amount of one-tenth of one percent may be used in the preparation of oleomargarine. Toxicological or chemical substances may be natural, as just indicated, or they may be "man-made." Doctor Yeary states: "Approximately 275 different man-made chemical substances, including drugs and antibiotics, are permitted in the feed and drinking water of food-producing animals. An additional 50 to 100 drugs may be administered to animals by injection or orally. Yet, of the substances that are considered drugs or pesticides, only about 17 are permitted to be present in meat, none are permitted in milk, and only two are permitted in eggs. . . . Well over 1,500 different chemicals are intentionally added to food."

Substances may be added to meat and meat-food products in definite amounts to act as antioxidants, emulsifiers, stabilizers, extenders, coloring agents, flavorings, flavor enhancers, tenderizers; to soften, emulsify, sweeten, improve appearance, increase water binding properties or alter natural characteristics of meat.

No food or chemical is safe or harmless or non-toxic under all conditions of possible use! All may consider to burden the metabolism upon ingestion.

In order for a chemical to be considered for incorporation in meat or meat-food product, it must first be shown to have need in the preparation of the product and to serve a useful purpose. Secondly, it must be shown it is safe and non-harmful. No product shall contain any substance which impairs its wholesomeness and which is not approved. Proof rests with the industry or proponents. If it is safe for use and if it will not produce a change in meat that makes it unsafe when consumed, the conditions under which it may be used are determined. It may be excluded from certain foods or certain labeling features may be required. Acceptable analytic methods must be established if a residue remains in the product.

Today's livestock is born and raised in an environment continually exposed or subjected to animal and plant pesticides, fungicides, herbicides, nematocides, defoliants, plant growth regulators, antibiotics, hormones and other growth regulators, tranquilizers, organic arsenicals, and many other veterinary drugs. In our complex modern technological society, the health hazards in food are no longer limited to specific zoonoses. Little wonder that the drug and chemical residues in our meat animals is great. The significance of this in human health is understandable. Chemical residues when ingested by man may be stored and released during a period of extended illness or weight loss so as to burden an already laboring metabolism. Sensitivity may occur and be made known, possible with disastrous results, upon medical treatment with the sensitizing antibiotic. Perhaps of greatest concern is that certain organic compounds that may have value in agriculture may also be potentially carcinogenic. This, however, has been inferred only from studies on laboratory animals and involved large dosage. However, many authorities hold the belief that there
is no threshold dosage for a chemical carcinogen—even a minute quantity of a carcinogen can produce cancer in given extended exposure!

New agricultural chemicals and livestock remedies are continually being marketed. The food hygienist must ever be on the alert to prevent the introduction into the food supply of harmful residues of these potent chemicals. The meat inspector must be ever aware of the agricultural practices in the area from which meat animals are received so as to be certain only wholesome meat reaches the consumer. There may be little if any reliable gross indications that a particular carcass contains such harmful residues.

However, in all fairness, let us point out that according to Doctor Radeleff,43 "Frank poisoning of chemicals in food of animal origin has not been observed..."

In 1961, the Food and Drug Administration surveyed foods purchased in grocery stores in Kansas City and Boston, for pesticides, herbicides, and fungicides. Foods representing a "total diet" were sampled and represented a composite of 12 major food groups. A very small percentage contained any detectable residue, and all were well within safe ranges. The pesticide levels were generally less than one percent of the legal limits.25

Doctor McCoy40 states: "The Meat Inspection Division has been conducting analyses for the detection of agricultural chemical residues in meat since 1947. Over the years we have found a steady decline in the amounts of residues present. This indicates an increasing awareness on the part of farmers and stockmen of the need for greater care in the use of these compounds of high biological activity. We anticipate the trend will continue."

Indications are that the measurable amounts of DDT and its metabolite DDE in human fat show no significant directional trend in body storage levels between 1950 and 1962.60

Chemicals and drugs that are of concern from a residue standpoint may be divided into three basic groups:

1. **MEDICATED FEEDS AND THERAPEUTIC AGENTS.** Antibiotics, hormones, anthelmintics, tranquilizers, arsenicals, etc.

   These agents are used for treatment of disease to increase rate of growth and efficiency of food conversion and to enhance livestock production by reducing mortality and production losses or retarded feed consumption and growth due to undefined infection.

   **Antibiotics:** The use of antibiotics as a preservative for extension of shelf life of meat, meat by-products or meat-food products is prohibited. Residual levels of antibiotics in meat resulting from the therapeutic or prophylactic application of the drug prior to slaughter should not be so high so as to have a significant preservative effect. The use of antibiotics or chemotherapeutic agents for the above purposes give rise to such healthwise dangers as:

   a. The occurrence of resistant pathogenic and non-pathogenic bacteria. Bacteria become non-susceptible to an antibiotic that
normally would have a lethal or inhibiting effect on them. It is known that small doses of antibiotics present as residues in foodstuffs cause, when constantly consumed, resistant bacteria in the intestinal flora of man. This could bring about resistant strains of such pathogenic bacteria as salmonella, staphylococcus and some of the coli type and render present day therapy useless. Bacteria are known to pass this resistance genetically during reproduction. Recently, newspaper accounts have related work indicating immunity to antibiotics can be passed on to related and non-related bacteria in some manner simply through near contact or proximity! Federal authorities are "taking another look" at the use of antibiotics used in human medicine and their use in livestock application lest in the future the bacteria in animals capable of infecting humans develop resistance and not be amenable to therapy when involving humans seriously. The Food and Drug Administration is re-studying the use and usefulness of certain broad spectrum antibiotics used in the fish and poultry industry to control bacteria growth and so extend shelf life. Antibiotics can mask spoilage and be used as a substitute for good sanitation. Antibiotics inhibit some spoilage bacteria and allow others and their toxins, not harmless, to grow. Thus natural spoilage characteristics are absent or concealed (odor, color, etc.), although the product so preserved may be grossly mishandled or held without proper refrigeration.

Unfortunately, meat inspectors are experiencing an increasing number of antibiotic injection marks or pockets of antibiotics lesions in carcasses. They are ever on the alert for these "tell-tale" signs.

b. Antibiotics, particularly penicillin, in food may induce allergic responses in some sensitive consumers. Antibiotics may alter the normal bacterial flora of the intestinal tract of individuals. Doctor Brune states: "Animals slaughtered after a treatment with antibiotics may show residues in the meat and organs of only a few parts per million. Work has shown when these tissues are examined bacteriologically, the results may be negative in the usual incubation time; but if the incubation time is extended, there is a positive result showing that pathogenic bacteria may be retarded in growth but not inhibited. This could present a real danger to public health."

c. Production of toxic symptoms. Chloramphenicol may be toxic to man. It may adversely affect the bone marrow to produce serious defects in blood cells. Streptomycin may affect the eighth cranial nerve associated with hearing. Chloramphenicol is not recommended for use in meat animals.

Hormones: Diethylstilbesterol is the most commonly administered hormone to increase weight gains. Its use is permitted in beef and
sheep. Diethylstilbestrol implants are forbidden in hogs. When fed in the ration, the hormone must be withdrawn at least 48 hours prior to slaughter. Implants should be restricted to the subcutaneous area of the ear and not injected indiscriminately into deep tissues.

Progesterone and/or testosterone propionate combined with estradiol benzoate may be used on lambs, steers and heifers but not used within a 60-day period before slaughter.

In some laboratory animals, diethylstilbestrol has demonstrated carcinogenic properties but this tendency has not been demonstrated in humans. However, one cannot be certain that the continued intake of synthetic estrogens by humans would not lead to malignant changes in susceptible tissues under hereditary conditions. Some malignancies in the human female may be stimulated to further growth by minute amounts of estrogens. Infants and children before puberty produce hardly any estrogen substances and are very susceptible to these agents. There is no indications a residue will remain if a minimum of 48 hours elapses between feeding and slaughter. These hormones are not destroyed by cooking. In several countries, estrogenic hormones for fattening livestock or for other nutritive purposes are forbidden.

Anthelmintics: The use of thiabendazole is permitted in cattle, sheep, goats, and swine but is not administered 30 days prior to slaughter for food. Cadmium oxide and cadmium anthranilate are permitted to be used in hogs but a 30-day discontinuation period is required.

Arsenicals: Incidences of frank and chronic poisoning of livestock with arsenic and other agents as lead and mercury are not rare to the meat inspector. Meat from affected animals was recognized as a potential danger since the inception of Federal meat inspection in 1906. Organic arsanicals are widely used as an anthelmintic and as a prophylactic growth stimulating feed additive in swine. Perhaps as many as 50 percent of the meat hogs have been fed arsenicals! Doctor Yearly states it is the only chemical used in food production known to be carcinogenic for man. It must be removed from feed five days before slaughter to permit arsenic residues to be eliminated from the animal. Arsenic: appears universally in nature and naturally in food-stuff. Arsenic is present in milk and in body tissues. A tolerance of 0.5 ppm is acceptable in meat and one ppm in meat by-products.

Mention should be made of phenotheizine. Phenothiazine is a potentiatior for the cholinesterase inhibitor, that is, the organic phosphate insecticides. Untowards results may ensue from phenotheizine treatment of animals that have been treated with grubicides, all of which generally used are organic phosphates.

Tranquilizers: As well as being capable of imparting residues, tranquilizers may mask symptoms of illness at ante-mortem inspection. Symptoms of disorders of the central nervous system may be suppressed. Unmanageable animals may be subdued with unauthorized tranquilizers shortly before being subjected to transit or
slaughter. This can only result in condemnation of the carcass. The production of wholesome meat requires that an animal be physiologically normal when presented for slaughter.

A second group of chemicals and drugs that are of concern to us from a residue standpoint is:

2. PESTICIDES. Prior to the publication of "Silent Spring" by the late Rachael Carson, the public was vaguely aware and little concerned with the toxicity of pesticides and the dangers they hold for people.

The presence of chemical residues in meats was recognized early enough in most cases to allow for study and resulting reasonable controls.

The introduction, use and application of a chemical in the agricultural food chain must be evaluated in terms of whether the benefits derived from its use in all cases equate or exceed that of possible hazard to man.

The so-called "Pesticide Amendment" to the Federal Food and Drug Law enacted in 1954 contained legislative provisions aimed at the protection of the consumer in connection with the occurrence of pesticide residues in food. This improved existing laws in that specific methods of controlling residues of pesticides chemicals in or on raw agricultural commodities was set up. Also, pesticide-chemical residues must be safe from the standpoint of the consumer.

Many of the existing pesticides are persistent on treated crops, in treated plants and in treated animals. Yes, persistent in Nature! They are highly resistant to decay and may literally "pop up" many hundreds of miles from the source of application on plants and on earth! Newspaper reports have dealt with the finding of pesticide residue in penguins and crab-eating seals in Antarctica—thousands of miles from land areas where pesticides are used in quantity! Minute quantities in our food are considered a potential hazard to our well-being when these chemicals may build up in our system as a result of continued ingestion as a food residue!

The chlorinated hydrocarbon insecticides (DDT, BHC, lindane, heptachlor, chlordane, dieldrin, aldrin, toxaphene, etc.) present the greatest threat and hazard. They accumulate and persist in the body fat. The organic phosphates are less of a threat in as much as they are rapidly eliminated and have a decidedly lesser tendency towards deposition.

For in-plant use, "knock-down" sprays containing pyrethrum extracts and other non-toxic material not having residual killing action may be used in rooms where meat is handled under certain restrictive procedures that protect food, utensils and equipment from contamination. Insecticides with residual activity are restricted to those areas where exposed meats are not handled. It might be added severe restriction and limitations are placed on the type, use, manner and area of application of rodenticides in meat establishments.

The Manual of Meat Inspection Procedure of the United States
Department of Agriculture lists pesticide chemicals and their established tolerance levels in meat and whether accepted for use on slaughter animals and on agricultural premises.

Some major conditions contributing to the adulteration of meat with pesticides are the unwarranted use of certain chemicals on animals and, principally, the disregard for labeling instructions!

Pesticide residues in carcass meats leave no scars; no "tell-tale" signs. They cannot be detected generally, through organoleptic signs. Again, our inspectors must know the agricultural "chemical climate" in which he lives, works, and from which the food animal originates. He must be able to recognize signs and symptoms of pesticide exposure and toxicity in the live animal.

During the processing of meat and meat-food products, there are approximately 100 substances, chemicals, preparations or compounds permitted to be added in definite amounts under certain conditions to certain food products. Some, like the nitrates, are frank poisons if ingested to excess. However, their security and safety is guarded by lock and key and by very close inventory control. All these items have shown need and safety under prescribed usage and conditions. Our inspectors are unrelenting in the enforcement of prescribed rules for use and untiring in their vigilance for possible advertant or inadvertant incorporation of unauthorized substances to meat-food products or even to their entrance on the premises!

Incidental or Indirect Additives: Our inspectors are ever mindful of and alert to the possibilities of these articles finding their way into our meat items. Acceptance of all detergents and washing compounds and their manner of use is required. Lubricants for food processing machinery is subject to acceptance as the paints lest substances, toxic in nature contact food and contaminate it. Plastics, coatings, adhesives, printing inks, and the like, must be first acceptable and not impart any unwholesomeness to food. The re-use of any container not lending itself to sanitizing is prohibited. Measures are applied to diminish the incidence of tramp metal, wood splinters, glass fragments, shavings, porcelain or enamel chips from finding their way into food.

Now we arrive at the second classification of meat-borne diseases:

THE ENDOGENOUS (INTRAVITAL) ANIMAL INFECTIONS TRANSMISSABLE TO MAN BY MEAT

These are for the most part zoonoses. Most zoonoses are at least potentially meat-borne and may be acquired through the "handling" of the meat or by its ingestion. These pose a threat to the handler of livestock, the slaughterhouse worker, and the consumer.

Some of the principal meat-borne helminth zoonoses are:

Trichiniasis: Trichiniasis attacks more than 25 mammalian species including man. Clinical cases which have been studied in Europe and in
the United States have largely been traced to consumption of pickled, smoked, fresh or improperly cooked pork. As a public health hazard, trichiniasis is limited for the most part to the Northern hemisphere—in particular to Europe and the United States. A survey conducted by Doctor Gould (1945) revealed an incidence of infection of human diaphragms of 16 percent. The ratio for years of life was one positive case to each 130 years of life before 1910 and one per 800 years of life after 1910! Dr. Kenneth MacDonald, School of Medicine, Iowa University, developed data from 570 human autopsy samples. This indicated the incidence in man has been reduced from one positive human case for each 126 years of life for people living prior to 1910 to about one human case for each 965 years of life since 1910. Recent reports indicate that less than four percent of human muscle samples now contain trichina and during the past ten years the number of clinical cases reported to the United States Public Health Service has been 225 and the deaths from trichinosis have been reported at four per annum. During the four-year period from 1944–1948, an average of 400 cases of human trichinosis were reported annually. The above figures point out significant improvement in the lowering of the incidence of trichinosis in humans—and the decrease applies also to infections in the swine population. Doctor Zimmerman relates a decrease in garbage-fed hogs from 11 percent in 1950 to 0.5 percent in 1964–1965. A further indication of the reduced incidence of T. spiralis in swine was revealed by the same author in studies made of fresh pork sausage. For the period of study ending in 1946, 12.6 percent of the samples were found to contain trichinae while in the study concluded in 1960, the incidence was one percent.

The reduction in the incidence in swine may be attributed mainly to the requirement of cooking of all garbage fed to hogs, a decrease in the number of garbage feeding premises, improved livestock husbandry sanitary methods and less trichinae in pork scraps.

Our American method of preventing trichinae infected meat from reaching the consumer is the treatment of pork, pork products, or pork containing product to assure destruction of the parasite. In Germany, the microscopic examination of muscle tissue from each hog carcass for T. spiralis is mandatory. Passing a carcass that may have a light infection is deemed to be unimportant as an infection rate of less than one trichina per gram of muscle tissue does not constitute a public health problem. Microscopic examination of tissues from each carcass does not lend itself readily to the American assembly line method of hog slaughter and processing. All hogs in the United States are not slaughtered under veterinary supervision. A false sense of security would occur engendering, perhaps, the consumption of raw or inadequately cooked pork. Federal and our New York State regulations require pork, pork products, or pork containing products that may be customarily eaten without further adequate cooking, be treated or subjected to an approved method to destroy the trichina organism. Pork, pork products or pork containing products that may have the appearance of being ready-to-eat must also be adequately treated to kill trichinae. Incidentally, the flesh from hogs
fed uncooked slaughter-house offal may be considered unwholesome. Heat application at 137°F and above readily destroys the organism as does low temperatures over a period of time as well as the salt-cure and slow, low temperature drying method. High "dosage" X-ray intensity is needed to kill trichinae but considerably less is required for sterilization of the organism to render it innocuous. The irradiation method is not used commercially.

Increasingly important in trichinae control surveillance is the large growing demand for "convenience foods" as frozen dinners, breaded pork products, partially smoked or cooked meats or sausage served in the home after a short period of cooking at relatively low temperatures.

The meat inspection services are ever vigilant to prevent possibly infected pork from reaching the market. Such common practices as beef for hamburger following pork through the grinder without first removing all traces of pork is discouraged and prohibited as is the adulteration of hamburger or chopped beef with pork.

*Taeniosis:* Tapeworms of the genus *Taenia* were among the first parasitic worms recognized in man. Hippocrates describes *Taenia saginata* and its association with excreted segments. Two species of *Taenia* may be found in man: *T. solium* and *T. saginata*. The former is commonly referred to as the pork tapeworm; the latter as the beef tapeworm. In the mature state, both species live in the small intestine. Man acquires the infection upon ingesting insufficiently cooked meat containing the larvae, cystercerci, which develop in the musculature of cattle and swine. The life cycle of each is maintained by contamination of the feedstuff or water of cattle and swine by human fecal material containing ova which, when ingested, develop into cystercerci larvae in the muscles of cattle and swine. When the infected beef or pork is eaten by humans, the viable cystercerci develops into mature form which then shed ova. So, the cycle is perpetuated.

According to Doctor Stoll's estimate about 2.5 million persons harbour *T. solium*. Auto-infection with the eggs of *T. solium* can take place in man. *T. solium* is clinically the more harmful, because of its propensity for developing cystercerci in human tissue outside of the musculature; that is, the brain, liver, and lungs. Doctor Faus states: "Human cystercercosis is rare in the United States and Canada, but rather frequent in Latin America and India...pork tapeworm infection is rarely encountered in the United States, but beef tapeworm infection is widely distributed in this country and elsewhere and further shows no evidence of diminishing in the human population." According to Doctor Ciolfi, in the United States those areas which are recognized as being heavily infected are located close to the Mexican border. In most countries the lower economic group and those who like to eat their meat rare are more apt to be infected with *C. bovis*. Cystercerci like *Trichinella spiralis* is rapidly destroyed by heat at 137°F or above.

A careful physical examination is performed on each beef and pork carcass for evidence of "measly" beef and "measly" pork. Unlike trichinae, cystercerci cysts are readily visible to the eye. The presence of
Cystercercus bovis is more often located in the heart, muscles of mastication, the diaphragm, tongue and esophagus; Cystercercus cellulosae in the pig's heart or tongue.

Disposition of meat found on carcass inspection to be infected with C. bovis is dependent on the number of cysts found, color and condition of the meat. Carcasses may be passed for food after removal of cyst and surrounding tissue and they may be refrigerated for a specific time at a specific temperature or they may be thoroughly heated throughout to a temperature of 140°F. "Carcasses of hogs affected with tapeworm cysts, Cystercercus cellulosae, may be passed for cooking; but if the infestation is excessive, the carcass is condemned."45

Hydatid Disease: The optimum definite host for human hydatid, Echinococcus granulosus, is the dog. Domestic and wild ruminants are usual intermediate hosts for the larval stage or hydatid which develops in the lungs or liver following ingestion of ova from an echinococcus infected carnivora. Ova ingested from fecal contaminated fingers or food polluted with dog feces may develop into a hydatid cyst of the human lung or liver. Man plays an incidental role as a blind alley intermediate host. Human hydatid disease is not acquired directly by consumption of infected meat. Meat can serve as a vehicle for the echinococcus ova. In certain areas of the world where the relationship of man–sheep–dogs is very close, echinococcus in man is endemic and measures are necessary to rid dogs and livestock of the parasite and to prevent re-infection by discontinuing the feeding of viscera and offal to dogs.

Organs or parts of carcasses found to be infested with hydatid cysts are condemned and destroyed on post-mortem inspection.45

Livers infested with flukes or fringed tapeworms are also condemned for human use.45 Sheep carcasses affected with tapeworm cysts (Cystercercus ovis, sheep measles—not transmissible to man) may be passed after removal and condemnation of affected portions. However, if infestation is deemed to be excessive making removal of cysts impractical, the entire carcass is condemned or it may be passed for cooking after removal and condemnation of the affected portions according to the degree of infestation.45

A third classification of meat-borne diseases can be made under:

INFECTIONS AND INTOXICATIONS DUE TO EXOGENOUS (HUMAN AND ENVIRONMENTAL) CONTAMINATION OF MEAT AND MANUFACTURED MEAT PRODUCTS

This phase deals primarily with the intoxications and infections liable to be conveyed by meat and manufactured or processed meat products contaminated after slaughter and during processing with human, animal or environmental pathogens or their products. This is, of course, primarily bacterial food poisoning.

Among responsible individuals in authority, there has emerged a growing awareness of the importance and need of veterinary public health measures in protecting people against illness by diseased meats.
Meat technology has developed rapidly in recent years and public health problems have increased in scope and variety due to the introduction of new sophisticated food products containing varied ingredients whose very nature and source may introduce new challenging facets to microbiological and chemical problems.

Regulations Governing Meat Inspection of the United States Department of Agriculture read: "All carcasses of animals so infected that consumption of the product thereof may give rise to food poisoning shall be condemned..." Authority is also granted to condemn in whole or in part any product previously inspected and passed that may be found to have become "unsound, unhealthful, unwholesome, or in any way unfit for human food..."45

The major trend towards centralized preparation and far reaching distribution of "convenience foods" makes mandatory specialized handling under adequate refrigeration to avoid contamination and subsequent growth of health hazardous organisms and their toxins. This makes mandatory eternal vigilance in preparation and distribution. One break in the sanitation chain can prove disastrous to many people over a wide area.

Doctor Foster asks, "How serious is the problem of food-borne diseases?" He goes on to state: "The entire question of food-borne diseases has been likened to an iceberg. We see only a small part of it above the surface. Reporting food-borne diseases is not mandatory in this country and there are no reliable statistics to show the true incidence.... Poultry and meat products have caused about half of the food-borne outbreaks reported since 1956 to The Public Health Service.... Estimates of the true number of food-borne diseases offer figures ranging from several hundred thousands to a million cases per year!"

The number of cases appear to be on the increase. Some individuals attribute this to the increased consumption of ready-to-eat foods prepared in processing plants. The food industry people state it is due to people calling their physician for minor complaints, as well as better diagnosis and education by public health officials.

The manufacturing of meat-food products does lend itself to adulteration; to the use of meats that have become perhaps unsound through improper handling and contamination. This more so than do "red meats;" "red meats" are much less incriminated as a source of food poisoning than meat products.

Over 100 zoonoses are listed but admittedly some are rarely found in humans. However, among the emerging diseases certain of the zoonoses are assuming greater proportions and importance as a result of changing ecological patterns.

The meat inspector concerns himself with the many public health aspects of meat processing. Doctor Clarkson lists some of the major ones:

a. Prevention of parasitic conditions transmissible to man from meat products (Trichinosis is an example).
b. Prevention of adulteration with uninspected, unclean, or unwholesome meats. For the fiscal year, some 23,000,000 pounds of meat that had been passed on ante-mortem and post-mortem were
condemned on reinspection because of later deterioration and destroyed. Certain aspects of adulteration and contamination may be difficult or impossible to detect in the finished product.

c. Prevention of adulteration with unsafe and unfit additives. Additives must first be proven to be safe, useful and to have value in the product before being acceptable. Such practices as the use of dyes to improve color of meat is prohibited as is the use of antibiotics. Antibiotics may mask spoilage. They are no substitutes for sanitation and adequate refrigeration.

d. Prevention of adulteration with filth, insects, rodent excreta or other foreign material.

e. Prevention of the use of adulterants that reduce nutritional value. Water and excess cereal are common extenders that may be used to excess in order to spare more expensive meat protein. Laboratory analyses is not an acceptable effective substitute for "on-the-spot" inspection during processing procedures.

f. Prevention of unsanitary conditions or procedures in the food plant and in the manufacturing process.

SELL IT OR SMELL IT!

Such was the by-line of industry until relatively few years ago. With the advent of new discoveries and methods of manufacturing, handling and packaging; the food hygienist must be alert to new hazards.

Doctor Dack11 states that the use of plastic wraps, impermeable to air, and the procedure of vacuum packaging may prolong the storage life of a meat item by preventing the growth of mold and certain bacteria which normally limit its shelf life. The added storage time may permit the growth and toxin formation of Cl. botulinum. An example of this is the outbreak of Type E Cl. botulinum from smoked Ciscos (Lake Superior fresh-water fish). Doctor Schmidt, et al.54 have indicated that Type E Cl. botulinum will grow at common refrigeration temperatures and produce toxins (3.3°C for six weeks).

In the opinion of Doctor Foster,27 the implication that vacuum packaging is unsafe because it may increase hazards of botulism is unwarranted. He states further that it has been shown that packaging in the absence of air has no significant affect on the ability of Cl. botulinum to grow in a food. The interior of most meat foods is anaerobic regardless of the method of packaging. Doctor Foster28 indicates the primary hazard in frozen foods, frozen pre-cooked foods, and catered food lies in contamination and perhaps microbial growth during preparation and processing. Equally important is failure to observe proper temperature control during storage, distribution, retailing, and in the home of the consumer. Growth is likely to occur at any time the temperature remains between 50° - 150°F. Catered foods are the ones most likely to be held long enough at this range to become dangerous. Mildly processed meat and poultry items that have been given a light bacteriocidal treatment destroys most of the natural contaminants that might grow and cause spoilage. If food poisoning
organisms happen to be present, or later become a contaminant, they may multiply and thus establish a highly dangerous condition.

Doctor Brandley relates in the 1930's when the practice of curing hams by injection of pickle via the femoral artery and then smoking and heating at a minimum low temperature of 137°F. was adopted by the industry, a number of staphylococcal food-poisoning outbreaks occurred in association with these hams. The heat killed the vegetative microbes. When chance contamination with the enterotoxigenic staphylococci occurred following the heat treatment, the staphylococci would grow readily without any competition. This resulted in a number of staphylococcal food poisoning cases due to such hams. This pointed up the necessity for proper refrigeration and handling. Such hams have not been a significant problem since.

Illness resulting from Cl. botulinum toxin has occurred from the consumption of meat and vegetable pre-cooked frozen pies when normal bacterial flora was eliminated or reduced allowing growth and production of Cl. botulinum with toxin production.

"All that Smells Does not Kill.
All that Kills Does not Smell."

Doctor Dack writes: "Food-poisoning outbreaks may be caused by one of a variety of etiological agents. Certain bacteria or their metabolic products are the most important of these agents."

The chief source of micro-organisms concerned in meat borne outbreaks are:

1. The human intestinal tract (salmonellae, shigillae and related species).
2. The human nose, throat and skin (staphylococci and streptococci).
3. The soil (Clostridium botulinum).

Agents causing bacterial food-poisoning may be divided into:

a. Preformed toxin producers as: Cl. botulinum, enterotoxigenic Staphylococcus aureus.
b. Living organisms as: salmonellae, shigellae, Cl. perfrigens streptococci (Alpha type), vibrio, B. cereus.

Doctor Brandley states: (1) "It now appears that humans are more likely to be the source of enterotoxigenic staphylococci than are lower animals."

Doctor Foster writes: "Staphylococcus poisoning is the most common food-borne illness in this country...not all strains produce the toxin that causes food poisoning. Food poisoning cultures produce an enzyme called "coagulase" although many coagulase producers apparently do not cause food poisoning. Nevertheless, it is common practice to look for coagulase positive staphylococci in foods as an indication that toxigenic strains may be present... Staphylococcus aureus is most likely to grow and produce toxin in foods that have been treated to inhibit or eliminate
other bacteria. This applies to cooked foods, as baked ham, roast fowl, and potato salad where there is opportunity for recontamination after cooking." No case has been identified in the United States as staphylococcal food-poisoning due to the development of staphylococcal enterotoxin in unpasteurized meats. For bacterial growth and toxin formation, the food must be held at a reasonably warm temperature (50° - 150°F.) for several hours. Toxins may be formed and accumulated under stop-and-go-growth conditions. Toxins are extremely heat resistant but the organism is readily destroyed by pasteurization or common cooking temperatures. It can grow in a concentration of salt and sugar, on cured meats, ready-to-eat meats, in poultry and in poultry dressing. A pH below 4.8 to 5.5 inhibits growth. Growth is discouraged at temperatures below 44°F. Refrigeration does not diminish the potency of the toxin.

Staphylococci are common contaminants of the skin, wounds, nose and throat of man. It is impossible to entirely avoid contamination. The inspector's concern is to reduce this contamination to a minimum. He is concerned with the maintenance of normal bacterial flora in cured and fresh meats and he is further concerned with the proper application of heat or cold in all phases of food manufacture and handling so as to prevent contamination and growth of harmful bacteria.

Botulism: The toxin is the most deadly poison known to man. Doctor Foster states it is the rarest, fortunately, but the most deadly! One gram of pure toxin might be enough to kill as many as 1,000,000 persons and incidents are known wherein people have died from the mere tasting of a spoiled string bean! Poisoning is caused by Clostridium botulinum Types A, B, Ca, Cb, D, E, and F. The toxin is preformed and is in the food prior to consumption. Cooking or boiling does destroy the toxin easily. Most cases of poisoning in the past were associated with home cooked canned vegetables. Spores exist in the soil and are natural contaminants. Abundant growth of Cl. botulinum with toxin production occurs usually under conditions where potential competitors have been removed. Types A, B, and E have caused almost all of the botulism in man.

In the case of Type A and Type B, Clostridium botulinum growth in meat products is accompanied by gas putrefaction. Type D has been reported in animals in Africa and an outbreak reported in a man in Africa was traced to raw ham stored in a defective refrigerator. The ham was normal in taste and appearance. Several outbreaks involving fish have been attributed to Type E Cl. botulinum. This organism, unlike Type A and most B, produce spores of low heat resistance. It will grow at refrigeration temperatures and is non-proteolytic. Whether Type E Cl. botulinum will be a problem in poultry and in the ready-to-eat meats is doubted by Doctor Dack, but he believes more information is needed by the ecology of this species in nature. In this regard Doctor Dack cites the work of Doctor Dolman and Doctor Iida. Doctor Brandly states: 'It is now generally recognized that the vacuum packaging of smoked fish does not promote the growth and toxin production by the Cl. botulinum Type E organism. Rather, vacuum packaging prevents the growth of molds and other strict aerobic spoilage organisms which would cause the food to
spoil in the absence of refrigeration. This extension of shelf life permitted the growth and toxin production of the botulinum organism. Ready-to-eat meats, such as canned hams, have not been reported to be the cause of botulism poisoning. This perhaps may be attributed to few spores contaminating the product; the applied heat and salt may inhibit growth of sporulating anaerobes. Canned hams do have spoilage spore forming anaerobes to act as indicators of abusive handling.

**Salmonella - "Refrigerated Poison"**: Of the living organisms causing bacterial food poisoning, the dreaded rising spectre is genus Salmonella. In Dr. James H. Steele's opinion, it is the principle zoonosis problem in the United States and the world today. In the United States it is second only to the staphylococci as a source of food poisoning. Doctor Kampelmacher states: "In post-war Europe, salmonella infection constitute a major health problem."

Salmonellosis is on the rise! From 1942-1963, the number of human cases of salmonellosis reported annually in the United States increased from 504 to 15,390! A total of 21,113 isolates of salmonellae from human sources were reported to the Salmonella Surveillance Unit in 1964. This represents an increase of 13.2 percent over the previous year. *Salmonella Typhimurium* and *S. Typhimurium var copenhagen* were again the most common isolates from both human and non-human sources. *Salmonella derby*, however, accounted for one-third of the human deaths, of which there were 57, associated with salmonellosis. The very young, the old, and the debilitated are the most seriously affected by salmonella poisoning.

Frequently, today's epidemic involves a large number of people who are exposed to the same vehicle of infection although they may not live in close geographic proximity. This probably results from modern marketing practices which allow for the widespread distribution and consumption of common food. Salmonellae inhabit the intestinal tract of both warm-blooded and cold-blooded animals and is composed of over 900 recognizable serotypes according to Doctor Edwards. All members of the genus are considered to be potentially pathogenic. With very few exceptions, the salmonellae colonize all species of warm-blooded animals with equal facility. The major reservoirs of human salmonellosis are in domestic livestock and the organism is believed to be transmissible from one to another or are derived from the same source according to Doctor Galton, Steele and Newell.

Numerous studies demonstrate continuing infection among large numbers of domestic and wild animals, and it is reasonable to believe that continuation is due either to a natural cycle in the host, or that there is some feedback mechanism from infected persons, other animals, food, or the environment. Evidence suggests that the main reservoirs of salmonellae is in animals and that the organisms are transmitted to man either directly from animal or through contaminated products of animal origin. Transmission to man is secondary to the continuing cycle in animals.
The reported frequency of salmonella isolations from fowl indicate that domestic poultry probably is the largest single reservoir of this organism among animals. More than two-thirds of approximately 20,000 cultures isolated from animals between 1934 and 1963 were derived from domestic fowl.\textsuperscript{20}

Salmonellae in the lymph glands of normal hogs and in retail pork products has been observed.\textsuperscript{58,7,31,32,57} Salmonella goes through the animal and may become resistant in the stockyard areas. Meat, bone, and feather meal teem with the organism.\textsuperscript{37}

Doctor Dack\textsuperscript{14} relates, "Unfortunately meat products from infected animals are often but not always the cause of salmonellosis."

The incidence of salmonellosis increases in the summer time and results from the ingestion of contaminated processed foods. Refrigeration curbs but does not kill the organism—thorough cooking does!

The prevention and control of salmonellosis should start with processing methods which will eliminate viable salmonellae from the product. Rendered animal by-products can be processed to be free and to remain free of salmonellae by providing separation of plant processing department and the personnel from the area where raw materials are handled. Product must be stored to prevent contamination from insects, rodents, and birds. As long as contaminated feed is fed, there can be little hope of improvement in the situation. The elimination of highly contaminated holding lots would markedly decrease the incidence of salmonellae. Time interval between departing from farm and slaughter should be minimal. Doctor Dack\textsuperscript{13} indicates there is abundant evidence that salmonellae are infrequently found in the intestinal tract of animals at the farm level. Thorough cleaning, proper heat and cold application, and strict sanitation greatly reduces the incidence of salmonella in the final product. Reducing the incidence among domestic animals and poultry would offer the best means and hope of excluding salmonella from areas of food preparation.\textsuperscript{23}

The education of food handlers at all levels and the effective regulating of food handling practices are important aspects not to be overlooked in controlling salmonellosis.

Meat inspection services are well aware of the significance and importance of the rapidly rising increase in the incidence of salmonellosis in our livestock population and in our food products. Such recognition and importance is given to the public health hazard of salmonellosis that the Regulations Governing Meat Inspection of the United States Department of Agriculture\textsuperscript{47} provide for condemnation and destruction of the carcass of an animal showing signs of salmonellosis.

Perhaps not as significant or dramatic in their impact upon human health are some of the other "performers" in the entourage of food-poisoning culprits. Singularly, they occur sporadically among the cases of food poisoning but collectively they add up to or account for a substantial number of such outbreaks or cases annually. Some of the uncommonly encountered organisms are listed in nodding recognition of their existence and importance in this subject:

\textit{Clostridium perfringens}. Normal inhabitant of the intestinal tract of man, animal and in the soil. It often finds its way into food. Some spores
are highly resistant to heat and may withstand prolonged cooking temperatures. Upon re-warming or exposure to warmth, the organism may grow prolifically and be infective when consumed, causing an enteritis. There are no changes in the physical appearance or nature of the food. Cooked meat is often incriminated. Again, proper refrigeration of cooked meat is a necessary safeguard.

*Streptococcus faecalis*, *Alpha Type Streptococcus*, *Bacillus subtilis*, enterococci, and *Vibrio parahaemolyticus* have been incriminated in foodborne poisonings associated with contaminated meat products, fish, dairy and poultry products.

**Viruses:** The possibility or potential of virus transmission in food has created new and growing interest recently. A wide range of viruses, not all pathogenic, may occur in our foodstuff. Doctor Lemon\(^3\) indicated the agent of Avian Leucosis and Bovine Lymphosarcoma are apparently present in the tissues derived from the host species and are transmissible to animal and perhaps to man. The greatest hazard may not be to the consumer but the livestock handler!

Now, what is the significance of microbiological findings or numbers in meats? According to Doctors Brandly, Migaki and Taylor: "The fact that hamburger made from the perfectly sound trimmings from aged beef commonly will be found to contain 60 million microorganisms, often shocks the individual unfamiliar with the presence of organisms in food.... Fresh meats, and particularly ground meats may develop very high numbers without organoleptic change and without any increased health hazard. It has been recognized for many years that it is often impossible to associate bacterial numbers with organoleptic change. Considering decomposition, there is no method of evaluation of acceptability for meat as accurate as organoleptic examination performed by one experienced in the subject. Therefore, it has been found impractical to establish bacterial numbers as a criteria for decomposition of meats.... It must be concluded that man's contamination of food and his subsequent mishandling of it accounts for almost all of the food-borne illnesses."

Only passing mention has been made of such infectious diseases as Tuberculosis, Anthrax, and Brucellosis capable of afflicting man. The incidence of these diseases now stem more frequently, perhaps, from direct animal and meat contact than through meat ingestion in this country. In this regard, these diseases and others as leptospirosis and listeriosis are more of a direct threat to the farmer, the animal handler, the slaughterhouse worker, the meat cutter, the meat inspector and the veterinarian individually than the public in general.

Our population continues to shift from rural areas to urban and suburban areas. New generations have come into being that are not aware of the deadly hazards which made for the original development of food hygiene practices and animal disease eradication or control programs so essential to animal and to public health. However, there is growing public knowledge of the hazards associated with large scale food production, processing and distribution. It is at these points new meaning and emphasis is being given to protecting the wholesomeness of our food by the meat inspection services.
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THE ROLE OF MEAT INSPECTION IN A DISEASE REPORTING SYSTEM

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The veterinary services of the States and of the Federal Government impose a relentless campaign against livestock disease, which benefits an industry which is notable for its productivity and animal health. There is danger that the better bred, better fed animal, raised in an environment free of specific pathogens may suffer severely from epizootic infections. The livestock industry still suffers from anthrax, anaplasmosis, respiratory diseases, neonatal infections, psittacoid abortions and other endemic problems. Faced with the threat of foreign animal diseases, there is good evidence we do not understand or control some diseases already with us. The erosive influence of these isolated instances tends to separate the livestock owner from the veterinary services, unless the situation is handled wisely, and the disease is suppressed. An invaluable part of disease control is the record of the morbidity and mortality together with the epidemiological and ecological features involved in the outbreak. The separation of the veterinary services into various functional bureaus, agencies and divisions has made total morbidity and mortality reporting more difficult. The crosswalk still exists, and the meat inspection services, local, State and Federal, are in the front line in reports of animal infections and conditions. There is need to explore the capability for greater participation in meaningful morbidity and mortality reporting.

The gathering of statistics is a burdensome effort unless there is a financial or health reward as a result. Efforts to express morbidity and mortality in the past sometimes face the criticism that the statistical calculations have been applied to the whole population, without regard to the probability that the majority of the herds throughout the nation were not actually at risk. Although reports during epidemics (anthrax, hog cholera, equine arthropod borne encephalitis) may seem exaggerated, even this number can be insignificant on a national scale. Serological surveys are equally subject to fault finding, as there may be a poor correlation between the positive animal and those actually diseased. Yet it is obvious that livestock losses are very important to the livestock economy.

The meat inspection services, responsible for the wholesomeness of poultry and red meats in a competitive situation where costs are a serious consideration, are unlikely to welcome any additional utilization of time. The choice is either to use present data, or to establish a cooperative program with other agencies, such as blood collection for brucellosis control, 6-35 traceback for tuberculosis, and trichinosis surveys. In addition, meat inspection services provide biological material and pathological specimens for the research and diagnostic efforts directed at numerous diseases. The records of these studies could become a part of morbidity
and mortality reporting system. One of these efforts is the study of tuberculosis lesions by laboratory methods.

Since the beginning of Diagnostic Services, at the National Animal Disease Laboratory at Ames, Iowa, the meat inspection services have participated in the collection of tuberculosis lesions. It was already known that grossly the tubercular lesion could be confused with those caused by a variety of etiological agents, requiring microscopic study for dependable differentiation. During the 1950's, advances in the field of human tuberculosis indicated soil or saprophytic acid-fasts cause lesions and induce tuberculin sensitivity. The eradication of communicable bovine disease will require ecological studies to eliminate infection by soil organisms.

A series of one hundred of these MI submissions indicates the meat inspector has continued need of laboratory support for a diagnosis of tuberculosis in non-reactor cattle. Although the number of non-tubercular lesions is a low 24 percent, it indicates the continued need for microbiological study of tubercular lesions. The pathologist found a 34 percent compatibility between gross and microscopic lesions, without microbiological isolation of acid-fast organisms. The isolations of 19 percent Runyon group acid-fast exceeded a 16 percent incidence of *Mycobacterium bovis*, but the presence of *M. avium* in seven percent pushed the balance to the side of the pathogens. Data related to these studies is given in Figure 1.

| Isolations of *Mycobacterium bovis* | 16 |
| Isolations of *Mycobacterium avium* | 7 |
| Isolations classified by Runyon scheme. | |
| Group II | - 2 |
| Group III | - 2 (resembles *M. avium*) |
| Group IV | - 14 |
| Not Classified | - 1 |
| Lesions only, no isolations | 34 |
| Lesions other causes, not tuberculosis | 24 |
| 100 |

Figure 1. Results of National Animal Disease Laboratory study of 100 tuberculosis lesions found by meat inspection.
Figure 2. Distribution of acid fast infections on a soil-probabilities map.

The acid-fast infections of soil origin appears to be ecologically restricted to alkaline soils, as the deviator problem is prominently identified with such soil areas. In 1959, a soil probability map for the occurrence of deviators was introduced for ecological study. The possible value of this approach is illustrated in Figure 2 with case numbers for the record. Although *Mycobacterium bovis* infections are not restricted by soil environments, it is easier for the pathogen to be missed in a deviator area. Western soils under arid conditions tend to be alkaline (as is the water normally), and only those soil areas likely to harbor large numbers of deviators are indicated on the map.

The submission of acid-fast lesions for laboratory study is but one example of cooperation in knowledge of the distribution of disease. Research workers and regulatory officials continually watch meat inspection reports for evidence of disease of particular concern to their investigations and programs. Not infrequently, major investigations are launched as a result of meat inspection condemnations. Studies of bovine leukosis, avian Mycoplasma and avian leukosis are recent examples of these effects. A major effort of this type is shaping up related to hog cholera.

In our efforts to eradicate hog cholera, the report of suspected hog cholera from meat inspectors is obviously necessary. The meat inspector should keep in mind that salmonellosis in particular may cause similar lesions, and seek laboratory assistance to eliminate the Salmonella and related organisms. It is important for the meat inspector to realize that at the present level of eradication, every lesion suggestive of hog cholera is important to the program. Finding such lesions should be
reported promptly (as by telephone) to the appropriate officials in the State. Such reporting is important in establishment of the epidemiology of hog cholera outbreaks. A specific diagnosis of hog cholera for eradication purposes includes history, microscopic pathology, and one or more recognized laboratory procedures. The meat inspector needs to back his findings with laboratory confirmation in support of this total effort. The swine industry, the veterinary profession, and the health of the public would be well served by a definitive diagnosis by laboratory means of all cases suspected to be hog cholera. The effort must include differential bacteriological and virological techniques, which should then become a part of the morbidity and mortality reporting system.

Because of the high level of meat inspection in the United States, shippers withhold diseased livestock, or separate the normal from the sick. Rejected meat generally represents chronic conditions, with secondary complications, which has its origin in larger losses in the feed lot and on the farm. Apparently normal animals in groups of visibly sick animals diverted from slaughter may also be diseased. Like the bulk of an iceberg, statistics from meat inspection reflect a much larger loss. Some of these

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Figure 3. Cattle, whole carcass condemned, per 100,000.
figures might justify the laboratory effort to give meaning to morbidity and mortality reporting. Meat inspection, based on gross pathology, and with limited time to study the disposition of retained meat, may serve morbidity and mortality studies best as source material for study. Limited support of both research and regulatory efforts has been a meat inspection role for years. It must be kept in mind that as in the case of trichinosis surveys and tuberculosis, the evaluation of actual disease requires laboratory support. Does the national morbidity and mortality situation justify this effort and expense?

From the "Summary of Activities" of the Meat Inspection Division, the data in Figure 3 and Figure 4 indicates we might justify the effort. The actual carcass loss in 1965 was 248 - tuberculosis, 846 - arthritis, and 9,616 - pneumonia. Each of these figures have different conversion values, dependent upon age, specific cause, and other factors. This is reflected in the number passed after removal of affected portions. A fourfold increase of 1,110 - tuberculosis, a sixfold increase of 61,692 - pneumonia, and a tenfold incidence of 83,980 - arthritis indicates conversion factors to be considered in morbidity and mortality reporting. The malignant lymphoma statistics reflect the seriousness of this problem which

![Graph](image)

Figure 4. Cattle, carcasses passed after removal of affected portions per 100,000.
should be made a part of an ecological study by way of a uniform animal identification program.

Much has been made of the role of Mycoplasma in causing airsacculitis and other lesions of poultry, with research and control programs as a result. Despite the seriousness of these losses to the poultry industry, the public health significance is apparently negligible. A more serious look might be taken at poultry septicemia. The 1965 losses presented in Figure 5 show different situations in the Western and North Atlantic areas, as compared with the poultry producing areas of the South. A laboratory study, on a statistically sound basis, might give us a measurable insight in the relationship of infection and environment. It can be surmised that in addition to specific pathogens such as the Salmonella, Pasteurella and paracolon organisms, there will emerge a class of organisms causing disease with an environmental association not readily reproduced in the laboratory. The poultry industry, with a uniform feeding and breeding program, is the best industry for study of the role of environment and infectious diseases.

We might well wonder if the meat inspection services could give us a clean status on the basis of the lesions described in 1890 for contagious

Figure 5. Condemnation of young poultry for septicemia, per 1000.
pleuropneumonia. We do know the epidemic situation brought about by this disease has been eradicated from the United States. Are we as fortunate with other Mycoplasma organisms of cattle, sheep and swine, in the obvious absence of Mycoplasma mycoides. As in the case of tuberculosis, the submission of suspect mycoplasma lesions for laboratory study should be a responsible effort to maintain the freedom that we now experience. The description of contagious pleuropneumonia lesions is general for this group of organisms.

"In early cases, the lesions consist of distension of the inter-lobular connective tissue with lymph, infarction of a portion of the lobules, and pleuritis, with a great abundance of false membranes. In more advanced cases the lung is hepatized, different lobules showing different stages of inflammation. The spaces of the inter-lobular connective tissue are filled with lymph and the pleura is greatly thickened. The lungs are neither tuberculated or abcessed. The varigated color of the cut surface of lungs, to which the term marbeling is sometimes applied, does not appear in another type of lung affection."

Participation of Meat Inspection efforts in a morbidity and mortality reporting system can be simplified if they as well as other agencies use a standard numerical nomenclature. This is available in the "Standard Nomenclature of Veterinary Diseases and Operations," an effort sponsored by the National Cancer Institute and the College of Veterinary Medicine, Michigan State University. The Standard Nomenclature culminates an effort in this direction by the American Veterinary Medical Association, which published "A Basis for Nomenclature of Animal Diseases—Topographic Classification and Etiologic Categories" in 1955. It makes use of a four digit topographic and etiologic classification like that proposed to the United States Livestock Sanitary Association by Sorensen in 1960. In this day of rapid retrieval of data by machine, the value of the Standard Nomenclature in uniformity throughout the veterinary services is at once obvious.

For many years, Animal Morbidity and mortality has been subject of considerable interest. Some feel that either a total or statistically valid distribution studies should be made toward a realistic appraisal of the disease situation, but the cost of such an effort, backed by necessary laboratory diagnosis of the morbidity observed, may be prohibitive. A more fruitful effort may be the study of ecological factors to identify the reasons for this distribution. Meat inspection efforts provide us with comparative data, which with laboratory studies, provide a solid base on which to organize field programs. This data should be retrieved within a uniform Morbidity and Mortality Reporting System.
REPORT OF THE COMMITTEE ON MEAT AND MILK HYGIENE


The Committee on Meat and Milk Hygiene is continuing its study in depth of the following eight major areas—as set forth in its Report presented at the 69th Annual Meeting in Lansing, Michigan, October 25 to 29, 1965.

2. Control of Unwholesome, Condemned and Inedible Material.
4. Toxic and Biologic Residues in Meat, Milk and Poultry Products.
5. Bills to Amend Federal Meat Inspection Act and Extended Federal Meat Inspection. (Senate #2678 and HR 11670 - 89th Congress of United States)
6. Federal-State Collaboration and Liaison (Training - Conferences - Administration - Technical Procedures)
7. Continuing Evaluation of and Assistance to State Meat, Milk and Poultry Inspection Programs.
8. Public Information.

In addition to the eight areas enumerated above, the Committee proposes to add the following two additional areas for study:

1. Veterinary Specialization in Food Inspection and Public Administration, American College of Veterinary Public Service Practitioners (Veterinary Specialty Board)

I. PUBLIC HEALTH AND ECONOMIC ASPECTS OF MEAT, MILK AND POULTRY INSPECTION

The public health and economic aspects of meat, milk and poultry inspection continues to generate increased attention of your Committee. To provide appropriate study of these aspects the Committee finds it necessary to define specific areas of public health that are related to meat, milk and poultry hygiene. These include but are not limited to the public
health aspects of salmonellosis, trichinosis, cysticercosis, swine brucellosis, and other zoonotic diseases. The Secretary of Agriculture has recently issued a statement of policy discouraging the use of poultry litter as an animal food. This substance as an animal food can very possibly serve as a source of salmonella infection as well as toxicologic pesticide residues. Your Committee recommends that each state take appropriate action to prevent the use of poultry litter as a ration component for livestock. Included also in these surveys are the epidemiological studies structured from tuberculosis and brucellosis disease data gathered from the Animal Health Division "back tagging" trace back program. The Committee also encourages the investigation for the development of a complete national livestock identification system.

II. CONTROL OF UNWHOLESAME, CONDEMNED AND INEDIBLE MATERIAL

Surveys conducted recently by the Committee on Meat and Milk Hygiene, United States Livestock Sanitary Association and by the Council on Public Health and Regulatory Veterinary Medicine, American Veterinary Medical Association reveal that many states do not have statutory authority and/or regulations to control movement and insure proper disposition of dead, dying, diseased and disabled (4D) animals and those condemned and inedible materials of animal origin.

Such control is essential for the (1) protection of health and economic welfare of the consumer, (2) control and/or eradication of animal diseases and, (3) protection of the Meat and Livestock Industries from the operations of unscrupulous operators who would jeopardize the prestige of the Industry, and the orderly marketing of meat and meat products.

The Committee on meat and milk hygiene would be remiss in discharging its responsibilities to the United States Livestock Sanitary Association if it failed to make appropriate recommendations for the control of condemned and inedible material and the 4D animals.

The Committee on Meat and Milk Hygiene respectfully requests that the United States Livestock Sanitary Association recommend that each state that has not already done so, enact appropriate legislation and/or promulgate adequate regulations to control the movement of dead, dying, diseased and disabled (4D) animals; to prohibit the use of dead and dying animals for human food and to require their destruction for such use; and to condemn and require destruction in whole or in part of those diseased and disabled animals determined by thorough ante mortem and post mortem examination to be unfit for human consumption. To accomplish this the Committee recommends that the legislation require that all people involved in the handling and/or marketing of diseased and disabled animals be required to operate under a permit or license or other form of registration. This includes (1) operators of rendering companies, (2) livestock dealers, (3) livestock haulers, (4) operators of auction markets, (5) operators of plants slaughtering and/or processing for animal feed and, (6) operators of plants exempt from state, county or municipal inspection. The Committee further recommends that inedible and condemned
materials of animal origin that resemble human food be properly de-
characterized and/or denatured and those not resembling human food be
properly handled to prevent the spread of disease through the contamina-
tion of public facilities, roads, thoroughfares, etc.

To accomplish this the Committee also recommends that individuals
handling these inedible materials be required to operate under a permit,
license or other form of registration. These would include:

1. operators of rendering companies,
2. operators of mink ranches and dog kennels,
3. operators of zoos and captive wildlife farms,
4. operators of refrigerated warehouses,
5. operators of pet food manufacturing plants,
6. operators of slaughter plants exempt from state, county or munici-
pal inspection and,
7. brokers concerned with the sale of inedible animal by-products.

It is by no means intended that these licenses, permits or registration
cause or be a financial hardship on the individual or be an additional source
of revenue for the state. Such registration is intended as a means of con-
trol and enforcement of legislation regulating the movement of dead and
dying animals and inedible and condemned materials.

The Committee is preparing an appropriate Model Law for the con-
trol of condemned and inedible materials of animal origin and the control
of 4D animals.

III. MODEL STATE MEAT AND POULTRY INSPECTION LAW

The Model State Meat and Poultry Inspection Law is undergoing a
continuing study and updating. This Model Law is furnished the Federal-
State Relations Office, Consumer and Marketing Service, United States
Department of Agriculture and other interested agencies and individuals.
This Law serves as a guide in development and/or amending of state
meat and poultry inspection laws. Further, such a model assists in estab-
lishing uniformity in state statutes. Your Committee encourages all inter-
ested agencies and individuals to submit comments and suggestions for
the updating and revision of the Model Law.

IV. TOXIC AND BIOLOGIC RESIDUES IN MEAT, MILK AND POULTRY PRODUCTS

Increased interest and concern has been stimulated by greater sur-
veillance and sampling of carcass meats by the Livestock Slaughtering In-
spection Division, Consumer and Marketing Service, United States Depart-
ment of Agriculture. This surveillance revealed that there was a high
residue level in meat carcasses in some areas of the United States. The
Committee recommends that greater attention by the Veterinary Profes-
sion and livestock groups in the distribution and use of agricultural pesti-
cides and insecticides. In addition, each state meat and poultry inspec-
tion agency should further develop a system of surveillance for control of
REPORT OF COMMITTEE

use of these chemical agents. Cooperative exchange of information systems for biological assays, and surveillance methods between the Federal Meat and Poultry Inspection Systems and those inspection agencies of state, county and municipal governments is encouraged by your Committee.

V. BILLS TO AMEND FEDERAL MEAT INSPECTION ACT AND EXTEND FEDERAL MEAT INSPECTION

Action by the 89th Congress of the United States on Senate 2678 and HR 11670 introduced on October 19, 1965, into that legislative body, has not been taken, nor is action expected prior to adjournment. Considerable activity by the present Administration to introduce similar bills for legislative action can be anticipated in the next Congress.

VI. FEDERAL - STATE COLLABORATION AND LIAISON

(Training - Conferences - Administration - Technical Procedures)

The Committee recommends that collaboration between the Federal Meat and Poultry Inspection Services and the states be continued and further developed. The official in charge of each state meat inspection program has been designated by the Chief Executive of each state and appointed by the United States Department of Agriculture as the Official Collaborator for his state. These collaborators met with representatives of the Federal Meat Inspection Service, Consumer and Marketing Service, United States Department of Agriculture at four regional two day conferences in San Francisco, New Orleans, Philadelphia and Chicago, during May and June, 1966. Agenda of these conferences included presentations of mutual interest by representatives of both State and Federal inspection services. Range of topics included subjects on training, administration, professional and technical procedures and proposed legislation. In addition, regular meeting and frequent personal communications were held by the appointed state and the designated Federal collaborators. Your Committee recommends again that each state collaborator appointed as the Official Collaborator with the Federal Meat Inspection Service, be re-evaluated by his state and in consonance with his duties, responsibilities and capabilities in a Total modern statewide meat and poultry inspection program. Your Committee further recommends that closer contacts thru the designated Federal collaborator be made to develop an information source on matters relating to poultry inspection.

The Committee continues to encourage the United States Department of Agriculture to assist state meat and poultry inspection programs in the following areas:

1. Training
2. Guidance in professional and technical aspects of an inspection program
3. Providing technical and administrative publications and materials and
4. Organization of laws and regulations for meat and poultry inspection.

It should be noted that training methodology should include (1) on-the-job training for professional and non-professional personnel, (2) administrative guidance at the several supervisory levels in the Federal Meat Inspection System, (3) Specialty training in such as, biological and chemical laboratories, labels and standards, and equipment and facilities, classroom instruction at the Meat Hygiene Training Center in Chicago, (5) use and development of visual aids as prepared by the Federal Meat Inspection Service.

VII. CONTINUING EVALUATION OF AND ASSISTANCE TO STATE MEAT, MILK AND POULTRY INSPECTION PROGRAMS

The continuing study and evaluation of each State Meat, and Poultry Inspection Program has been intensified thru a formal and informal mutual exchange system. The American Veterinary Medical Association has cooperated in this data collection effort.

Your Committee has furnished pertinent information and data to a number in excess of 25 states requesting the same. Additionally, duplicate materials were furnished the Federal Meat Inspection Service for its use in assisting the States. The Committee will continue to assist states in their program development.

VIII. PUBLIC INFORMATION

Your Committee will endeavor to develop means by which information on meat, milk and poultry hygiene will be disseminated to all interested publics. The following named agencies, groups, societies and/or organizations are suggested as foci for distribution of such information:

1. Veterinary Colleges
2. Colleges of Agriculture
3. American Veterinary Medical Association
   a. Council on Education
   b. Council on Public Health and Regulatory Veterinary Medicine
4. State Veterinary Medical Associations
5. Meat and Poultry Industry Organizations
6. Livestock Organizations
7. Farm Organizations
8. Consumer Organizations
9. Agricultural and Home Demonstration Services
10. State and County Fairs
11. Animal Health Division, United States Department of Agriculture

Your Committee recommends the development and utilization of films and visual aids and information such as pamphlets, briefs and brochures to enhance participation in public relations. Also, use of radio and television media in the public relations effort is encouraged.
IX. VETERINARY SPECIALIZATION IN FOOD INSPECTION AND PUBLIC ADMINISTRATION

The Committee on Meat and Milk Hygiene recommends that a Veterinary Specialty Board be established for the area of food hygiene and inspection and public administration. The Committee suggests that this Specialty Board be designated as the "American College of Veterinary Public Service Practitioners." The total involvement of the Veterinary profession in this specialized field necessitates the recognition of competence required to permit efficient discharge of professional and administrative responsibilities. Increasing complexities as well as increasing types and numbers of inspection programs further demand such recognition. The Committee will prepare plans for the development of this Specialty Board in accordance with the Plans for Organization of Veterinary Specialty Boards, established by the American Veterinary Medical Association.

X. PREREQUISITES, SELECTION, TRAINING, DEVELOPMENT AND EVALUATION OF PROFESSIONAL AND NON-PROFESSIONAL PERSONNEL FOR MEAT, MILK AND POULTRY INSPECTION

Your Committee will continue to furnish assistance to develop curricula for the formal training of Veterinary inspection technicians. Such assistance will be rendered to Agricultural Colleges and other Institutions of Higher Learning and to the Council on Education and Council on Public Health and Regulatory Veterinary Medicine, both of the American Veterinary Medical Association.

Similarly, the Committee will develop guidelines for assistance in course design in Food hygiene in the Veterinary professional course. Assistance also will be administered for a curriculum for veterinarians pursuing graduate studies in the discipline of food hygiene and public service.

Guidelines will be established for the prerequisites and selection procedures for Veterinary inspection technicians. These guidelines will be based upon data gleaned from review of current and future demands and availability of this category of personnel.

Criteria for employee training, development and evaluation will be determined on results of the aforementioned review. This entire activity will be conducted on a continuing basis. Projections of future needs will continue to be this Committee's function.

The Committee recommends that the benefits of meat, poultry hygiene received by the consumer, the meat packer and the livestock owner be extended to cover all meat and poultry slaughtered in the United States. These meat inspection programs must be adequately financed and located in that agency of government which can provide adequate funds to support and can enforce all aspects of a Total modern meat and poultry inspection program. Such Programs should be staffed with properly selected personnel, properly renumerated and free of conflict of interest and other influence.
DEMODECTIC PARASITES IN LIVESTOCK
Donald W. Baker, B.S., D.V.M., Ph.D. and
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INTRODUCTION

The demodectic mange mite has been incriminated as a parasite of most of the mammalian host species of animals. In the extensive literature concerned with the parasitisms of animals and man, there is a paucity of information dealing with demodicidosis. In one of the best and most recent reviews, Dr. H. J. Smith (1961) noted that "much of what has been written is fragmentary." He also stated that "it would seem that this condition in large animals has been inadequately studied and, as a result, is not a well understood phenomenon." From our observations and studies of the past 30 years, we endorse this as an understatement of facts. Our studies, begun by the senior author in 1935, have been concerned mostly with the parasitism in cattle, goats, and sheep, and have yielded intriguing but inconclusive information, so that we have hesitated to publish even the opinions drawn from critical observations.

The demodectic mite has been known to be a parasite of man since the report by Simon in 1842. Studies carried on throughout the world have elucidated many of the interesting aspects of this parasitism, and the recent study by Spickett, which utilized in vitro culture as well as histopathologic techniques, is a significant contribution. He estimates that the life of the female, which includes egg, larva, protonymph, deutonymph, and adult female, will require a minimum of 348 hours or 14 1/2 days. He notes that in man there is no evidence to suggest that the mite ever occurs in tissues other than the hair follicles and their associated glands. He states that, "the precise course of the life cycle, with respect to the duration and respective habitats of each stage is not known. The method of dissemination is also unknown, though various suggestions have been made. These lacunae in our knowledge are unfortunate especially as this mite is of importance in certain human diseases."

Bovine demodicidosis was first reported in 1845, and was mentioned by Foxon in 1878 as a cause of damage to leather. More than a dozen published reports during the intervening years have mentioned the pathogenic effects of this mite as a cattle parasite. We consider that Smith's comment: "Seasonal or environmental influences on the development of demodicidosis in cattle are not well understood," to be another of his understatements.

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Demodectic lesions are given most consideration by the owner or animal handler when the animals are being fitted for show or sale. The parasitized animal's discomfort (pruritus), caused by the development of many skin lesions, is more apparent to the person who is attempting the best management of his charge in the show ring.

We hesitate to comment on the transmission of this parasitic disease, but the mites may be found in sweat glands, according to Baker and Nutting, and the meibomain glands in the eyelids are common focal sites. Russian workers have mentioned a seasonal incidence of the clinical parasitism, but since we have encountered well developed clinical manifestations in all seasons, we do not consider this to be valid in this country.

A digest of the literature indicates that there is a great variation in pathogenesis. Demodectic acariasis is given more consideration when it occurs as a complicating condition with certain bacterial and fungal diseases. It is always difficult to assess the importance of two or more pathogens in advanced cases, but it is probable that the arthropod parasitism preceded the other pathogens.

There are many economic considerations involved in demodectic mange of cattle, goats, and other domestic animals where the hide has commercial value. For many years the hide and leather trades have been disturbed by the apparent increase in incidence of this parasitism.

It is unusual for the casual observer to recognize the disease clinically in beef animals, while in dairy cattle it is fairly common and easy to detect signs of the parasitism. Tanners, on the other hand, are more concerned about damaged hides from beef cattle.

We are hesitant to indulge in speculation, but a careful consideration of the information presented by reliable reporters, and our observation during the past three decades, suggest that this parasitism, in an inapparent or subclinical form, exists generally in adults of all susceptible animal host populations.

MATERIALS AND METHODS

Our objectives in this study include an investigation of all possible avenues of exposure of the susceptible hosts, and the mechanism which converts the inapparent parasitism into a recognizable clinical disease. The ultimate objective is to develop methods of control and prevention which will lead to eradication of the disease.

Dr. Jacob Traum called our attention in 1939 to the fact that demodectic mite parasitism was involved in many skin disturbances of herbivorous animals where the etiology was not well established, and, during the 25-year interval, we have found that demodectic mange is the complicating if not the prime pathogen of skin disturbances of cattle in many regions throughout the North and South Americas, as well as in Europe, Asia, Africa, and the Philippines.

Biopsies from serious cases of dermatitis in cattle diagnosed as Streptothricosis (*Dermatophilus* spp.) almost invariably show the presence of demodectic mites in sections or scrapings.
Since our projected study required materials in the form of the specific parasites, and detailed clinical information about the parasitized host animal, we devoted much time and work to surveys of the livestock population of New Mexico.

It became obvious from the results of our preliminary surveys that the incidence of clinical demodectic mite parasitisms in cattle in this region was much less than in the regions covered by our previous 30 years of experience.

We invited the members of the practicing veterinary profession, including the personnel of the Federal and State livestock disease control agencies to aid us in the survey, and have received very cordial cooperation. The operators of the slaughtering and meat packing companies have been equally cooperative, as well as the dairy herd, and cattle feeding operations.

Using the facilities of these local institutions and the opportunity to examine the animals passing through the two larger regional livestock auction markets, we have used most of the past year carrying out the survey noted above. A few of the parasitized animals have been purchased and are kept on the research station where a constant surveillance is maintained in order to study the progress of the clinical disease. In other cases we have purchased the hides removed from animals showing well established lesions. We offered the ADP laboratory services for the examination of scrapings and biopsies from the hides of suspected animals with signs of dermatitis of unknown etiology to interested veterinarians and livestock owners.

The results of these laboratory examinations have aided the clinician in establishing specific differential diagnoses in some cases, and those with negative findings have eliminated the fungi, bacteria, and certain skin arthropod and nematode parasites.

The techniques used to demonstrate the arthropod parasites of the skin are simple. Material collected by expressing the exudate from nodules and from superficial skin scrapings is placed on a microscope slide, mixed with a few drops of oil, a cover slip is added, and the field is examined using the 30X for scanning, the 100X for verification, and the 450X for closer morphological studies.

In those instances where this inspection technique fails to demonstrate the mite parasite, and particularly where a large amount of material is available for examination, we resort to the maceration-flotation technique. Wherever the first cover slip preparation does not provide one or more of the demodectic mites, we replace the fluid, add another cover slip, recentrifuge, and frequently find mites even though the first film was negative.

The demodectic mite is easily recognized and differentiated from other arthropod species because of its unusual shape and small size. *Demodex sp.* is described as minute (167-400μ), vermiform (cigar shaped), with eight short, five-segmented legs as adults. The mouth parts are equipped with stylet like chelicerae and three segmented palpi. The male genital opening is dorsal in the center of the podosoma, while the female
genital opening is between or behind the last or fourth coxae. The female mite produces a large oval egg and the development of the embryo is similar to that of other arachnids. The legless larva develops into the nymph with eight legs and finally into the adult mite. All of these stages may be found in the contents of the nodules of the skin of cattle and goats.

In the publication (Barn Itch - 1946) the senior author described the exudate from the skin nodules as "thick, white, and of a waxy consistency." We have found that the easiest and best method for spreading this material on a microscope slide is to drop a small amount of ether or chloroform on the exudate. The waxy, sticky material incorporated with the parasites is dissolved, leaving a thin film of the material to be examined. The usual sites for the lesions (nodules) are the sebaceous glands and hair follicles.

A few of the reporters of bovine demodicidosis have attempted to estimate the population of mites in a lesion; these estimates range from a few to incalculable numbers. In a piece of tanned leather presented to us in 1966, we counted more than 1,900 mites in the material removed from one small defect (lesion). We were able to identify 365 separate lesions or leather defects in the skin covering the left shoulder and leg.

The distribution of the parasitism is cosmopolitan. We have observed clinical signs in cattle wherever we have traveled, and in 1951 the senior author found well developed signs in a group of two-year-old animals which had been deposited in a jungle region of eastern Peru where no wild or domestic species of livestock had lived for hundreds of years.

As noted by Smith, the disease is infrequently reported in young animals. In our own experience, we remember one six-month-old dairy calf, and O'Flaherty reported occasional signs in six-month-old calves. We have demonstrated mites from clinical signs in cattle more than 12 years old and in two Indian ponies which were declared to be more than 25 years old.

RESULTS AND DISCUSSION

We have demonstrated the demodectic mange mite in specimens secured from clinical examples of dermatitis in the horse, cow, goat, sheep, and pig. Some, suggestive of the Demodex acariasis, have been differentially diagnosed as Streptothricosis and ringworm, as well as the nematode Rhabditis and Stephanofilaria parasitisms. Some animals which had been provisionally diagnosed as having Psorergates sp. dermatitis were found to be harboring the demodectic mite. Many had to be listed as "dermatitis of unknown etiology." A summary of the results of this first year's study is appended. A digest of the information secured from this year's work suggests that:

1. The incidence of clinical signs of demodectic mite acariasis in New Mexico is marginal, and less than that present in the rest of the continental United States. Nearly all found in dairy cattle were identified as animals which have been imported from other regions.

2. We have obtained no conclusive data to support the idea that it is a seasonal disease.
3. The clinical parasitism has not been found in calves under one year of age.
4. The classical clinical picture of demodectic acariasis in cattle and goats, with nodules of various sizes scattered about the anterior part of the body from face to shoulder, has been observed.
5. In sheep and swine, the parasites have been found in scrapings of the skin on the back and abdomen.
6. The mites have been found in the skin in the region of the eyelids.
7. No mites have been found in the tissue around the mouth, nose, vulva, and anus, but they were found in scrapings of the prepuce from a parasitized Corriedale ram.
8. From the few number of carcasses of goats and cattle studied, there is no evidence to suggest that the mites migrate through the internal body tissues.
9. Comprehensive studies of the carcasses of stillborn goats failed to show any mites from body tissues or contents of the digestive system. No mites were found in the placenta or colostrum milk from a parasitized female goat which presented two full-term kids at parturition. Frequent examinations of the twin kids, from a female goat which continues to have viable mites from nodular lesions, have shown no evidence of demodectic mite parasitism.

In general, our findings support the conclusions and speculation of many reporters that there is a high incidence of the parasitism in the form designated as inapparent or subclinical parasitism.

Representatives of the Tanners' Council of America, and of several companies involved in the tanning industry have supplied us with information which indicates that there is an increase in the incidence of mangy (demodectic) hides supplied to the tanneries. They are particularly concerned with the problem of damaged hides from "heavier weight calf skins."

ACKNOWLEDGEMENTS

We are grateful to Drs. I. H. Roberts and W. P. Meleney, of the Animal Disease and Parasite Research Division laboratory, Albuquerque, New Mexico, for advice and aid, and to the operators of the two larger slaughtering establishments in Albuquerque, the Schwartzman Packing Company and the Karler Packing Company, as well as to the Lobo Packing Company which kills horses and processes horse meat products. Our neighbor, the Valley Gold Dairy, which operates a one thousand-milking cow ranch, has been most cooperative and has extended both encouragement and kindly interest in our study.

NARRATIVE SUMMARY

In the light of former observations and experience, we consider that the incidence of clinical demodectic mite parasitism of cattle in New Mexico is much less than in other regions of the country. However, a random
sampling of the native bovine population suggests an appreciable incidence of subclinical or inapparent parasitism. This phenomenon is also true for the native equine and porcine populations.

No Demodex infestations in the young of any class of livestock were found, except for goats. It will be difficult to secure animals of any species for use as adequate uninfested controls in investigations designed to demonstrate the mode and time of initial exposure. The dairy goat breeds provide the best subjects for use in all spheres of investigation of demodicidosis.

TABLE I
Examination for Demodectic Mites of Randomly Selected Farm and Laboratory Hosts in New Mexico - 1965-1966

<table>
<thead>
<tr>
<th>Host</th>
<th>No. Specimens Examined</th>
<th>No. Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>56</td>
<td>17</td>
<td>30.4</td>
</tr>
<tr>
<td>Swine</td>
<td>13</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Sheep</td>
<td>38</td>
<td>2</td>
<td>5.3</td>
</tr>
<tr>
<td>Horse</td>
<td>37</td>
<td>24</td>
<td>64.9</td>
</tr>
<tr>
<td>Goat</td>
<td>10</td>
<td>4</td>
<td>40.0</td>
</tr>
</tbody>
</table>

REFERENCES


The summaries of the 1966 fiscal year's activities in sheep scabies eradication, cattle fever tick eradication, cattle scabies eradication, and the status of the screw worm eradication program are being submitted for inclusion in the published proceedings of the United States Livestock Sanitary Association.

The Committee reviewed proposed draft for updating Title 9, C.F.R., parts 72, 73, and 74, to allow more adequate control over scabies outbreaks, establishment and maintenance of quarantines, movements into and out of quarantined areas, and reference to certain parasites not clearly defined in present regulations. The Committee recommends that the proposed changes be carried out.

The Committee further recommends that the scabies classification be extended to include psoroptic, sarcoptic, and psorergatic mite infestations in sheep and chorioptic, psoroptic, sarcoptic, and psorergatic mite infestations in cattle.

SHEEP SCABIES ERADICATION

Continuing Progress Made

In Iowa, where 119 infected flocks were found the previous year, concerted efforts, including two inspections of all flocks, considerable attention to the epidemiology of each outbreak, and greater stress of inspection and dippings at market centers, resulted in the disclosure of 32 outbreaks in the following counties: Buena Vista (2), Calhoun (1), Cerro Gordo (1), Cherokee (4), Fayette (1), Floyd (1), Greene (1), Hamilton (2), Henry (1), Humboldt (1), Lucas (3), Marion (3), Mitchell (1), Pocahontas (3), Ringgold (1), Sac (1), Taylor (2), Union (1), Van Buren (1), Wayne (1).

Although it was expected that additional outbreaks would be found in Iowa, the fact that 17 outbreaks were found in States considered relatively free of the disease emphasizes the point that considerable work remains before psoroptic sheep scabies can be eliminated from the United States. These outbreaks occurred in the following States and counties:

- Connecticut - Litchfield (1)
- Illinois - Champaign (1), Cook (1), Lee (1)
- Indiana - Greene (1)
- Kentucky - Christian (1), Trigg (1)
- Nebraska - Morrill (1)
New York - Allegany (1), Orange (1), Ulster (4)
Pennsylvania - Bucks (1), Lebanon (1)
Texas - Uvalde (1)

During the year 20,010,807 sheep received official inspections and 405,629 were dipped.

CATTLE FEVER TICK ERADICATION

Prevention—keeping the ticks out of the United States—is a major part of the effort against cattle fever ticks. A quarantine zone is maintained along the international boundary and the lower Rio Grande River in eight Texas counties as adjacent areas in Mexico are infested. Cattle from Mexico are carefully inspected for ticks at the border. They must be free of ticks and must be given a precautionary dipping before they can be imported. Without these controls, cattle fever ticks would reinfest areas of the United States that have warm climates.

Active Program Continued in Texas

During Fiscal Year 1966 in the Buffer zone, 273 livestock illegally crossing the border were caught, of which 52 were tick-infested; 22 tick-infested Texas herds were found; and 61,223 lots of 1,689,702 livestock were inspected and 14,039 lots of 86,613 livestock were dipped.

Tick Surveys and Exotic Ticks Found

During Fiscal Year 1965, 3,920 tick survey collections were made including 1,910 from cattle; 362 from dogs; 1,016 from horses or mules; 235 from zoo animals and miscellaneous; 320 from native wildlife; and 77 from animals offered for entry.

Exotic ticks collected from imported animals or materials or those offered for importation included Rhipicephalus evertsi evertsi, Boophilus decoloratus, Rhipicephalus pulchellus, Hyalomma marginatum, Rhipicephalus bursa, Rhipicephalus evertsi mimeticus, Rhipicephalus appendiculatus, Dermacentor nitens, Amblyomma hebraeum, Amblyomma dissimile, Amblyomma rotundatum, Haemaphysalis leachii muhsami, Amblyomma cruciferum, Boophilus microplus, Amblyomma tholloni, Amblyomma gemma, Amblyomma variegatum, and Rhipicephalus simus simus collected from an eland, a hartebeest, a giraffe, zebras, horses, rhinoceroses, snakes, boa constrictor, a bat-eared fox, an iguana, cattle hides, and an elephant in the States of New Jersey, Florida, Texas, California, Kansas, Maryland, New York, Michigan, and the Commonwealth of Puerto Rico.

STATUS OF THE SCREWWORM ERADICATION PROGRAM

In the Continental United States eradication of established screwworm populations has been achieved. A Barrier Zone is in operation along the United States–Mexico border from the Gulf of Mexico to the Pacific Ocean. Most of the sterile screwworm flies produced at the laboratory at Mission, Texas, are being released in northern Mexico to reduce screwworm populations in Mexico as far south of the United States as possible.

During calendar year 1966, 3,794,297,550 sterile screwworm flies
were released over the southwestern United States and northern Mexico. Of this number, northern Mexico received approximately 71.64 percent of the sterile flies.

During Fiscal Year 1966, there were 999 screwworm cases laboratory confirmed in the United States. 765 of these cases occurred inside the Barrier Zone and 234 outside the Barrier Zone as follows: Texas, 381 cases in 52 counties; New Mexico, 115 cases in 10 counties; Arizona, 487 cases in 11 counties; and California, 16 cases in two counties. Although the Barrier Zone has proven effective, an occasional gravid female fly manages to penetrate the sterile fly Barrier Zone and infest animals in the southwestern United States. Each time a sporadic outbreak is found, emergency measures are taken and to date no screwworm populations have been allowed to become re-established in the United States.

A survey has been conducted throughout the Republic of Mexico to determine the feasibility of moving the barrier to a location in southern Mexico where it can be more economically operated. The survey has disclosed screwworm populations throughout the Republic of Mexico. However, the populations predominate along the coastal regions.

**PSOROPTIC CATTLE SCABIES REPORTED**

Fifteen outbreaks of psoroptic cattle scabies were reported in three States during Fiscal Year 1966. In Iowa, a veterinary practitioner was doing other professional work in a dairy herd in Winneshiek County when he noted lesions indicative of scabies and notified regulatory officials. Psoroptic mites were identified 9/13/65 via utilization of the maceration-flotation procedure. This was the first case reported in Iowa since 1961 when additional outbreaks were also reported in Colorado, Illinois, Oklahoma, and Texas.

Seven psoroptic cattle scabies outbreaks were disclosed in California during Fiscal Year 1966. Outbreak No. 1 was reported in Yolo County on 7/23/65 when an instructor at the School of Veterinary Medicine, University of California at Davis, diagnosed scabies affecting a bull belonging to the School. Outbreak No. 2 was diagnosed in a Kern County feedyard 8/16/65 by a veterinary practitioner employed by the feedyard. Outbreak No. 3 was diagnosed on 12/9/65 during routine inspection of a Ventura County feedlot. On 2/9/66 a veterinary practitioner's report led to a diagnosis of scabies in a Merced County herd (outbreak No. 4). Outbreaks Nos. 5, 6, and 7 were diagnosed in Merced County on 2/14-15/66 as a result of extensive epidemiological investigations initiated by disclosure of outbreak No. 4. Four psoroptic cattle scabies outbreaks were reported in California during fiscal years 1954-1965 (Fiscal Year 1954 - 3 and Fiscal Year 1965 - 1).

Seven outbreaks of psoroptic cattle scabies were reported in Texas during Fiscal Year 1966. Outbreak No. 1 was diagnosed on 12/8/65 when psoroptic mites were isolated from one steer in a group of 253 steers trucked from a Hale County, Texas, feedlot to the South Memphis stockyards, Memphis, Tennessee. Mites were also demonstrated on cattle remaining on the premises in Texas. Outbreak No. 2 was diagnosed on 12/11/65 in a Castro County feedlot. Disclosure of the disease in feedlot
No. 1 initiated inspection of feedlot No. 2 as both feedlots are operated by the same family. This is the third outbreak of cattle scabies in this Castro County feedlot in the past two years. A veterinary practitioner’s report led to the diagnosis of outbreak No. 3 in a Hale County feedlot on 2/11/66. Routine inspection of cattle in a Federal quarantine area led to the disclosure of infection in a Castro County herd, outbreak No. 4, on 2/9/66. Outbreak No. 5 was diagnosed in a Hale County herd on 2/23/66 when the owner requested that his herd be inspected in order to move cattle out of a Federal quarantine area. Outbreak No. 6 was diagnosed by a Texas inspector at the Plainview Livestock Auction, Plainview, Texas, in a group of Randall County cattle on 2/23/66. Outbreak No. 7 was disclosed on 2/24/66 during inspection of cattle remaining on the home ranch of the owner of infected herd No. 6. Sixteen outbreaks of psoroptic cattle scabies were reported in Texas during fiscal years 1954-1965 (Fiscal Year 1954 - two, Fiscal Year 1955 - six, Fiscal Year 1956 - one, Fiscal Year 1959 - two, Fiscal Year 1961 - one, Fiscal Year 1962 - two, Fiscal Year 1964 - one, Fiscal Year 1965 - one).

With the exceptions of California outbreaks Nos. 4, 6, and 7, Iowa outbreak No. 1, and Texas outbreak No. 6, the outbreaks involve multiple possible sources and in a number of cases, cattle shipped from several States. Although extensive epidemiological work has been done, it was not possible to pinpoint the source of these outbreaks.

During the year, 22,509,512 cattle were inspected for scabies. An increase of approximately four million over the previous year.

RESOLUTIONS

SHEEP AND CATTLE SCABIES

Proposed by Committee on Parasitic Diseases and Parasiticides.

RESOLUTION ONE

WHEREAS, the accelerated sheep scabies eradication program has progressed to the point that the incidence of the disease has been reduced to its lowest level in the Association’s history, and

WHEREAS, the goal of complete eradication of the disease is yet to be reached,

THEREFORE, BE IT RESOLVED, that this Association and all regulatory officials exert every effort this winter to conduct an eradication program designed to complete eradication of the disease.

RESOLUTION TWO

WHEREAS, there has been an increase in the incidence of cattle scabies, and,

WHEREAS, there is a need to place continued emphasis on scabies inspection activities, particularly in areas involved in last year’s outbreak and areas where foci of the disease may still exist,

THEREFORE, BE IT RESOLVED, that this Association support extended inspection activities designed to locate and eliminate cattle scabies.
DRUG RESIDUES, SYNERGISM AND ANTAGONISM

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An extensive outbreak of a toxicologic disease in dogs was first reported in 1952. Later research associated this problem with a commercial dog food which contained peanut meal as a principal protein source. This disease problem in dogs was similar to the previously reported "Moldy corn poisoning" in swine. Extensive losses in turkeys in England in 1960 resulted in the loss of over one hundred thousand poults. Research stimulated by these losses led to the isolation of a group of chemical compounds, aflatoxins. These agents cause severe hepatotoxicosis in several species of animals and carcinogenesis in others.

Recent work by Newberne has shown conclusively that during the past several years much of the feed-grade peanut meal purchased on the open market has been contaminated with aflatoxins. Recent action by the Food and Drug Administration and the Food Industry has assured consumers that edible peanuts and peanut products will be carefully screened for such toxins or residues.

Cultures of Aspergillus flavus have been isolated from such protein concentrates in Florida that under proper conditions of temperature and moisture content produced aflatoxins causing similar symptoms and lesions in white peking ducklings. These have included weight losses, decreased feed consumption, and icterus. Post-mortem lesions included pathology of the liver, kidneys and heart. A research program is underway to determine whether disease losses of twelve to fifteen percent in swine in Costa Rico in the last two years may also be due to aflatoxicosis.

It has been demonstrated by Armbrecht that high aflatoxin levels could be produced by several strains of A. flavus when grown on wheat, buckwheat, oats, corn, rice, soy beans and peanut meal under favorable moisture and temperature conditions for mold growth. Mayne and others have recently reported that cotton-seed kernels and hulls were utilized as substrates by an aflatoxin elaborating strain A. flavus with the production of high levels of aflatoxins B₁, B₂, G₁, G₂.

A proper understanding of drug action on the several animal species necessitates knowledge on the different effects on these species, the formation of active, persisting, or toxic metabolites, individual variations in the metabolism of the drug or chemical and equally important, the possible synergistic or antagonistic effect of chronic administration of the drug on its action. Some drugs are characterized by tolerance, or decreased activity or toxicity because they stimulate their own metabolism at a constant dosage level. Thus, phenobarbital is recognized as being one of the

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best "enzyme stimulators." Other agents would include the pesticides, DDT and chlordane.

It was recently reported by Burns\textsuperscript{12} that the normal lethal dose for Warfarin for rats was 30 mg./Kg. Following exposure to DDT or chlordane, the previously toxic dose was no longer active. It now required 300 mg./Kg. to produce death. It was proposed that, perhaps, where rats are not being properly controlled by Warfarin that this may have been due to previous exposure of the rat population to DDT or chlordane. The rats, because of the "enzyme stimulators" are better able to metabolize the Warfarin, thus reducing its toxicity.

It is probable that these "stimulators" alter the microsomal enzymes and thus influence the activity of drugs. Again, phenylbutazone, phenobarbital, chlordane and DDT predisposed to microsomal enzyme activation and do influence toxicity studies. They may also influence the steroid levels in the blood stream, carbohydrate metabolism and even fertility. It has been suggested that the insecticides may even be serving as "antifertility" agents. Thus, where frequent and repeated treatments with the insecticides to control external parasites is practiced, the conception rate may be decreased. This area should be investigated very carefully because of the economic impact on those geographic areas where such practices are widespread.

Many drugs have been tested for safety, as well as efficacy on either normal animals or in those in which pure infections were experimentally induced. Under field conditions, there may be chronic or other predisposing conditions to alter both safety, efficacy, and toxicity. It is known that several of the frequently used drugs, including chlorpromazine, reserpine, and progesteronal agents may alter hepatic functions with modification of biliary flow.

Nutritional deficiency may enhance drug toxicity. Stress may predispose to drug toxicities and result secondarily in congenital defects. Physical or surgical organ damage may predispose to drug toxicity. Therefore, in addition to the influence of drugs on growth or production, other parameters, including blood enzyme levels, hematology, neuromuscular disturbances, behavioral changes and the effects of drugs on certain diseases should be examined regularly.

As indicated, certain diseases may potentiate a drug's toxicity; although an antibiotic may control a bacterial infection, it may predispose to fungal infections in the gastro-intestinal tract. Injection of certain of the fatty acids may predispose to thrombogenic activity; heparin and serotonin have been so characterized.

Carcinogenic agents have been described as locally active, primary carcinogens; pro-carcinogens or remotely active and co-carcinogens. The locally active agents act at the point of application in single or small doses and may be influenced by the activity or toxicity of the compound. They would include the triphenylmethane compounds and certain inorganic chemicals. Pro-carcinogens would include the carbamates, the aromatic amines and azo dyes. CCl\textsubscript{4} is broken down into CHCl\textsubscript{3} and may be carcinogenic. In certain species of animals, including the dog, liver and bladder
cancer are common after administration of 2-naphthylamine. Studies on new drugs and a review of those now in use should support the freedom from carcinogenicity in animals under normal or ordinary conditions, as well as the usual disease entities.

Because of the widespread interest by those in government and industry, much attention has been paid recently to the "zero or minimum tolerance" concept. As analytical techniques have become more refined and parts per billion can be detected, the "zero" concept has become unrealistic. Rather, safe, minimal tolerance levels should be established for the more than 200 pesticides now on the market.

Antimicrobial drugs have been used in the treatment of bacterial infections for about thirty years. As experience accumulates in the use of these agents, the extraordinary capacity of the microorganisms to circumvent the action of the antibacterial agent becomes increasingly apparent.13

The clinical and epidemiologic implications of episome mediated transfer of drug resistance are obvious. For example, ingestion of Esch. coli or other bacteria with transferable resistance factors might convert bacterial strains already present in the intestinal tract to drug resistant ones and such subjects might become carriers of drug resistant intestinal flora. Although transfer of resistance of such factors apparently occurs less readily in vivo than in vitro, transfer and emergence of resistant strains might be accelerated by the selective pressures of chemotherapy.

The use of cottonseed meal as a dietary source of protein for monogastric animals has been limited because of the sensitivity of such animals to gossypol. It has been previously shown that addition of iron salts to the diet in such animals reduces or eliminates the toxicity. The level of iron used has been relatively high and caused a dark discoloration of the feces of animals to which it was fed. Clawson14 reported that an iron to gossypol ratio of 1:1 was adequate to protect growing pigs against toxicity when up to 400 mgs. of gossypol per Kg. of diet were fed.

As new and more potent drugs are being introduced, one must not only know their action but also what possible dangerous complications may develop when used in conjunction with other drugs. Any unwanted reaction on the patient's part must be noted and countered either by withdrawal of the drugs or the use of suitable antidotes to overcome the crisis. Drugs requiring careful usage would include the antibiotics, anti-depressive, antihypertensive and steroidal agents.

The streptomycins and neomycin were noted to produce respiratory depression, a neuro-muscular block and a fall in blood pressure similar to that produced by magnesium ions. Neomycin potentiates the neuro-muscular blocking effect of curare and succinylcholine; this action could be reversed with neostigmine. Other antibiotics, including polymyxin, bacitracin, and kanamycin, have similar actions. Cases of respiratory depression, even apnoea, have been reported and these are resistant to treatment. Thus, none of these antibiotics should be used until the relaxant effects of anesthesia have completely worn off.

It was recently reported by Belam15 that treatment of patients with steroids has the effect of decreasing the activity of the adrenal cortex by
suppressing the pituitary secretion of ACTH. Patients become euphoric, there is a redistribution of body fat, some glycosuria may develop, there is a decreased response to any infective process, tissue healing may be delayed and long-term therapy may lead to osteoporosis. The effect may last up to one year. Such a patient, when presented for anesthesia, is at hazard since their response to stress is absent or minimal. This may be marked by profound hypotension, coma, and death. Administration of extra hydrocortisone prior to and immediately following surgery is essential.

While the use of the synthetic organic insecticides has resulted in a marked increase in crop production and decrease in the incidence of insect borne diseases, at the same time there has been a growing concern regarding the effects of the distribution of such quantities into the environment. The Food and Drug Administration has established standards concerning the maximum amounts of pesticide residues permitted in or on foods such as fruits, vegetables, meat, dairy, and poultry products. Urban atmospheres may become contaminated from three sources: (1) Outlying agricultural areas where drift from sprays or dust may occur; (2) Local insect control operations; and (3) Industrial plants.

Samplings for individual insecticides present in the air were made in a number of representative cities in the United States. Measurable amounts of DDT were found in 17 of 20 samples analyzed and of aldrin in seven samples from Florida City, Florida. Chlordane was found in 14 of 16 samples collected at Lake Alfred. In one community relatively high concentrations of DDT were found and persisted for several hours after fogging. Malathion was also found.

Before total airborne pesticide concentrations can be determined and potential physiological or toxicological effects on humans, animals and plants evaluated, suitable methods must be developed for the simultaneous collection of both aerosol and vapor forms and analysis of the resulting samples.

Abrams repeats the two major factors of concern in studies of new drugs, that is, safety and efficacy. He stresses the importance of animal toxicity trials for predicting or avoiding side effects in humans. This includes acute, chronic research and since 1957 reproduction experiments for teratogenesis. Individual human reactivity, however, is not always predictable from animal studies. These reactions may affect certain organs, liver, kidneys, or hemopoietic system; the activation of peptic ulcers by the cortico-steroids, phenylbutazones, salicylates; hemolytic reactions to the antimalarials, sulfinilamide and nitrofurantoxin; bradycardia in reserpinized patients under anesthesia, etc. We are all familiar with the photoallergy seen in animals following the use of phenothiazine, chlor-tetracycline, griseofulvin and other agents. Clinical investigators, therefore, must seek to identify side effects as vigorously and methodically as they seek to identify efficacy.

Finally, Hunter recognized that exposure to chemicals occurs during embryonic life, at birth, during growth and development, maturity and reproduction, senility and at death. However, he stresses that the great
hazards to health are the insufficiencies. For the last fifty years we have lived in the age of chemicals; now, we enter the age of toxicology. Exposure must be reduced to well below any known threshold limits for injury. Figures in the United States show that the average concentration of DDT in human fat is 10 ppm. and cow's fat 2 ppm. This latter concentration provides a constant source of DDT in cows' milk and thus higher concentrations in milk drinkers. Farmers and veterinarians have a higher proportion of antibiotic resistant bacteria in their nasal passages and drug resistance can be transferred. Perhaps, injury may accrue to men and animals when chemicals are metabolized in their bodies, the metabolic processes themselves causing excess enzymatic activity, organ proliferation, metaplasia and neoplasia.

**SUMMARY**

1. Certain molds produce toxins which may persist in food stuffs predisposing to pathologic changes, carcinogenesis or even death.
2. Drugs or their metabolites may markedly alter their own action as well as potentiate or decrease the action of other drugs.
3. Nutritional deficiencies or mixed infections in animals under field conditions may predispose to increased toxicity of drugs found to be safe and effective in preliminary laboratory trials.
4. Determination of safe, minimal tolerance levels should be established for the more than 200 pesticides now on the market.
5. The clinical and epidemiologic implications of transfer of drug or antibiotic resistance is most important.
6. Steroids may predispose to a decreased defensive response to infections, tissue healing may be delayed, anesthesia may become much more hazardous.
7. Airborne insecticide contamination poses a hazard to both men and animals.
8. Drug residues in man and animals may predispose to excessive enzymatic activity, organ proliferation, metaplasia and neoplasia.

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ANTIBIOTICS: TRANSFER OF RESISTANCE, EFFECTIVENESS, AND CURRENT USAGE

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The infective hazards of intensive livestock farming, combined with the widespread nonmedical use of antibiotics and other chemotherapeutic agents, and the abuse of these agents at so-called "preventive levels" favor at one and the same time the spread of bacterial infection and that of drug resistance in certain pathogenic organisms.

The future of the anti-infective drugs may be short lived due to increased resistance. There has been an apparent increase in the number of bacterial organisms demonstrating some form of antibiotic resistance. It would be unfortunate if the benefits of the antibiotic drugs were limited unnecessarily.

Bacterial resistance may result from (1) the selection or survival of a minority of originally resistant bacteria through the destruction of other sensitive strains, such as penicillinase-forming staphylococci; (2) from a change in originally sensitive strains, usually through genetic or chromosomal mutation or adaptation; (3) by a recent finding that bacterial drug resistance can be transferred by bacteriophages from resistant bacteria to susceptible bacteria, and (4) as a result of cell to cell contact with a concurrent passage of cellular material. This transfer of resistance, which takes place extra-chromosomally, may occur between various strains and species.

Bacteriophages, viruses which attack bacteria, transfer the ability of bacteria to resist the antibiotic drugs bactericidal or bacteriostatic effects to the other sensitive bacteria that have not been exposed to an antibiotic.

The passage of cellular material via cell to cell contact may allow resistant E. coli to pass its resistant properties to antibiotic sensitive Salmonella, Klebsiella, Pseudomonas or Shigella organisms. Further, the bacteriophages of newly resistant organisms may transfer the resistant properties causing antibiotic sensitive bacteria to become antibiotic resistant bacteria without the newly included resistant bacteria ever being exposed to the antibiotic drug involved in the development of the original resistance. Some researchers are concerned of the fact that the transference of resistance goes hand in hand with multiple drug resistance or the development of an all-encompassing type of cross-resistance among anti-infective drugs. The ability of a single antibiotic drug to perpetuate antibiotic bacterial resistance to another unrelated antibiotic drug has been established. For example, if an organism becomes resistant to tetracycline, the resistance may be perpetuated for an indefinite length of time by the presence of any other single antibiotic or chemotherapeutic agent such as neomycin, streptomycin, sulfonamides, penicillin, etc. The
ramifications of "infectious drug resistance" are illustrated by the following example reported in 1965.\textsuperscript{4} \textit{Salmonella typhimurium} type 29 was originally sensitive to ampicillin, chlorotetracycline, kanamycin, neomycin, streptomycin, oxytetracycline, tetracycline, and sulfonamides; however, resistance to streptomycin and sulfonamides appeared in 1963, to the tetracyclines in early 1964, and to ampicillin in late 1964. Under these conditions, once a resistant strain becomes established by this mechanism all of the commonly used antibiotics in veterinary medicine will favor the persistence of the resistant bacteria.

It has been reported that antibiotics in animal feeds have led to bacterial drug resistance.\textsuperscript{5} The resistance is dependent on the size of the bacterial populations involved and the possibility of selective multiplication of mutants. Additional factors include the type of antibiotic drug and the period of time during which the bacteria were exposed. Experiments have shown that resistance exhibited by \textit{Escherichia coli}, \textit{Salmonella typhimurium}, and \textit{Staphylococcus aureus} may increase considerably with the amount of antibiotics used.

Tetracyclines used as animal feed additives may lead to the appearance of resistant strains of intestinal bacteria.\textsuperscript{6} The practice of feeding diets containing tetracycline to pigs and chickens almost invariably resulted in the emergence of \textit{E. coli} fecal flora predominantly resistant to tetracycline. The tetracycline resistant \textit{E. coli} persisted for a long period of time in herds of pigs after tetracycline had been discontinued. An increase in diseases associated with \textit{E. coli} were noted in herds of pigs in which tetracycline feed had been used.

Attention is drawn to the apparent decrease in the sensitivity of certain bacteria to streptomycin as reported in England.\textsuperscript{7} In 1957, 93 percent of the streptococci were susceptible to streptomycin whereas in 1960 only 44 percent were susceptible. Similar percentages of susceptibility for 1957 and 1960 for the tetracyclines were: coliforms 78 percent and 32 percent, Proteus 40 percent and zero percent, and Pseudomonas groups 33 percent and zero percent, respectively. Predominantly sensitive bacterial populations were replaced by resistant populations.

The rate of resistance to tetracycline and chloramphenicol among Salmonella strains was investigated in the Netherlands.\textsuperscript{8} Approximately 77 percent of all resistant strains encountered in 1961 were found among the human pathogen \textit{Salmonella typhimurium}. In 1961, cross infections caused by tetracycline resistant \textit{S. typhimurium} were observed in man and in calves. In a recent report, the number of isolations of resistant \textit{Salmonella} has increased.\textsuperscript{9} In man, calf, pig, and other animals, 12,472 strains belonging to 18 serotypes were isolated. A considerable increase in resistant strains was noted: nearly 11 percent as compared to two to four percent in previous years.

The role of antibiotic animal feed additives in developing resistant organisms in man and animal has been reported.\textsuperscript{10} It was found that antibiotic resistant strains of bacteria were much more prevalent in animals maintained on diets containing antibiotics than in animals maintained on diets that did not contain antibiotics. Likewise, resistant strains were
found in much higher number in the animal caretakers or farmers on the farms using antibiotic feed additives than on those farms not feeding antibiotic feed additives. Phage typing and other tests conducted on samples obtained from the farmers were usually identical with samples isolated from their animals.

There is no doubt that bacterial resistance to antibiotics and other chemotherapeutic agents has been established. The observed increase in resistance must be attributed to usage and misusage of this group of drugs. The anti-infective drugs properly used—discriminantly, judiciously, intelligently—could prove a powerful means of therapy for a wide range of bacterial diseases, and for a period of time which would greatly exceed that currently anticipated. The reduced longevity can be related to widespread indiscriminate practices. Little attention has been given to properly designed studies to determine antibiotic efficacy at subtherapeutic levels for disease conditions and certain nutritional claims.

Let us consider some of the common uses of the anti-infective drugs when used at levels less than optimum therapeutic levels.

The anti-infective drugs are widely used in animal feeds in the hope of improving feed efficiency, gain, and in reducing feed costs. This is a large market and is approximately $100,000,000 per year. In 1963, two and one-half million pounds of antibiotics were marketed for agricultural uses primarily as animal feed additives. It is often said by interested parties that antibiotic feed additives have long established their value when added to animal feeds; however, it should be noted that the corporate realization of the potential market for feed additives in the 5.5 billion dollar feed industry stimulated maximum promotion and sales energies.

To critically review data pertaining to the value of anti-infective drugs as feed additives is a job that requires accurate compilations of performance data which is not readily available.

Plumlee\textsuperscript{11} has reported on antibiotic drugs as growth stimulants for swine. He compiled swine performance data reported in Swine Day Reports from the Illinois, Indiana, Michigan and Ohio agricultural experiment stations. The reports cover a period of 1953 to 1962. The tabulation of data involved 46 experiments, 700 control and 2,150 antibiotic fed pigs. A summary of the data revealed that an increase of average daily gain was the most consistent finding, 14.7 percent for short term period and 6.9 percent with antibiotics were fed from weaning to market. Overall, a pig in this series of experiments which received antibiotic feed additives could be expected to grow faster and be marketed 6.9 or approximately seven days sooner than the pig that did not receive the antibiotic. The effect that antibiotics have on improving feed efficiency was found to be only a fraction of that claimed. The overall average percent of feed saved was found to be 1.53 percent which was not enough to offset the cost of adding the antibiotic. Not considering labor and capital investment aspects for a hog that can be marketed seven days earlier, an improvement of two percent in feed efficiency was needed as the break-even point. Feed cost per 100 pounds of grain for the 46 experiments showed that the addition of antibiotics to swine feed did not reduce such costs.
but actually increased the cost eight and one-half cents per 100 pounds of gain.

A 6.9 percent decrease in the time it takes to market a hog is a point that needs to be considered further. Such questions as: How much cost is associated with a 6.9 percent saving in labor? What effect does this saving in time have on capital investment? A pertinent question pertaining to herd health is: Are faster growing animals more susceptible to diseases? There are some indications that animals that grow faster than normal are more susceptible to some diseases. It has often been noted that animals on antibiotic feeds have thinner intestinal walls and some suggest that this improves absorption; however, one must also consider the possible reduction in the tissue integrity that may make it easier for diseases to develop, e.g., Salmonellosis.

In regard to antibiotic feed additives, when used at levels higher than that for growth promotion but below therapeutic levels, to prevent, aid or control animal diseases, one need not go very far before there is an abundance of claims stating that this is the most effective and economical method to handle animal diseases. Scientific data to document such claims are scarce; however, there is an abundance of testimonials and uncontrolled case reports. If it is true that anti-infective drugs when used at suboptimal therapeutic levels in feed aid, prevent, or control animal diseases, then because of their widespread usage, one would anticipate a reduction in the percentage of those diseases that respond to anti-infective drug therapy. However, when the United States Department of Agriculture disease incidence reports are checked, the exact opposite is found to be true for a period of 1960 to 1965. Atrophic rhinitis, arthritis, enteritis, mastitis, and pneumonia have all shown an increase in incidence at slaughter house and stockyard inspections. This should not be interpreted as being a sole factor attributed to antibiotics, good or bad, for other things such as management, feeding, etc., are also included; however, the lack of disease reduction does not serve as a case endorsing such antibiotic usage.

The problem must be considered in terms of the development of resistance and the efficacy of anti-infective drugs for nutritional and disease purposes when used at other than therapeutic levels.

The ability of bacteria to develop resistance to anti-infective drugs is documented in the scientific literature and the wide use of these drugs must be considered as a major contributing factor in the perpetuation and acceleration of bacterial resistance. Even when antibiotics are added at the level of 10-20 gm/ton of feed, it is possible that this amount is sufficient to induce resistant strains of Salmonella. All the prerequisites for subsidizing bacterial resistance have been fulfilled; widespread exposure, constant exposure, sub-therapeutic levels, close crowding of livestock populations, and diminished attention to sanitation and preventive husbandry practices.

The question of efficacy remains clouded. More reports designed similarly to that of Doctor Plumlee's are urgently needed. It would be desirable to review the efficacy of the growth stimulants in order to obtain
scientifically objective information regarding gain, feed efficiency, and cost per gain. From the data available, it appears that the benefit of nutritional feed additives may only be a fraction of what has been claimed. Experiments with blind placebos are needed.

In addition to the resistance problem associated with the use of antibiotic drugs, animals administered antibiotics represent a potential public health hazard. The public health hazard must be considered as antibiotic treated animals may serve as reservoirs for bacteria pathogenic to man and animals. They are also potential sources of residual antibiotics in the animal tissues and body fluids and thus may sensitize some people.

Some researchers feel that cattle are one of the most important, if not the most important, source of human Salmonellosis in Britain. The large-scale use of antibiotics and other antimicrobial agents in animal husbandry results in whole populations of animals being exposed to these drugs, often under environmental conditions in which the spread of enteric pathogens is inevitable. The authors stated that conditions which foster the selection and spread of drug resistant Salmonellae in livestock result in the appearance of the same antibiotic resistant organisms in man.

The fact that a certain portion of the human population may have undesirable reactions to minute amounts of antibiotics is well established. A common source of penicillin was found in milk. In the United States the incidence of antibiotic adulterated milk has dropped from 11 percent to less than one percent; however, in Britain in 1964, the examination of 41,700 milk samples revealed 11 percent contamination. It was noted that both urticaria and contact dermatitis was associated with penicillin adulterated milk and that symptoms disappeared in the intake of dairy products or milk was discontinued. In the United States the incidence of antibiotic contamination in milk has been well established in the past six years; however, the incidence of antibiotic contamination in meat, poultry, and eggs is relatively unknown.

Undesirable antibiotic reactions in cattle may be increasing. Brisbane reported 1,200 bovine reactions to antibiotics. Anaphylactic shock, urticaria, and local reactions were observed. The majority of the anaphylactic reactions were noted following penicillin or a combined penicillin-streptomycin injection. The highest incidence of undesirable reactions was observed in the autumn and winter months, coinciding with the handling and movement of large numbers of cattle for export. It was found to be a common practice to administer penicillin before and after transport.

The availability of information regarding the presence of antibiotic residues in slaughter animals in the United States is practically nonexistent except by sparsely limited nonpublished investigations. Antibiotic residue information has been reported in a few European countries. In Denmark, a urine antibiotic assay was investigated. The following positive results were obtained indicating the presence of antibiotic residues: cattle 12 percent, calves 58 percent, and swine 23 percent. Although the experimental sample size was quite small, the results suggest some degree of the problem.
In another Danish antibiotic residue investigation of meat, a larger number of animals was used. Urine was assayed to determine antibiotic activity in 1,000 cattle, 3,000 pigs, and 2,000 calves. Antibiotic activity was found in approximately one percent of the cattle, 0.5 percent of the swine, and 77 percent of the calves. The incidence of antibiotic residues may be related to the geographical area, type of husbandry, and the availability of antibiotics to nonlicensed, nonprofessional people.

In chickens fed five I.U. of penicillin or 10 μg of oxytetracycline per gm. of feed for six weeks, penicillin could be detected in the liver for five days and in the kidneys for six days after it has been withdrawn from the feed. Oxytetracycline could be detected in the kidneys for three days and in the caecum on the 12th day after withdrawal.

Pitre and Martinet reported an investigation involving many types of meat samples and found that 4.1 percent of samples contained a detectable level of antibiotic drugs.

SUMMARY

The current and future of antibiotic drugs for veterinary medicine and nonmedical agricultural use involves problems of bacterial resistance, potential animal reservoirs of infection to man and animal, and sensitization reactions in man due to antibiotic residues. The longevity of antibiotics as therapeutic agents may be reduced due to widespread nonmedical usage at subtherapeutic levels to large numbers of animals over long periods of time.

It appears that a scientific evaluation of efficacy, an assessment of resistance development, and monitoring system for detection of antibiotic residues in food is necessary in order to determine if current agricultural antibiotic practices should be restricted or curtailed.

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DRUG RESIDUES AND THE VETERINARY PROFESSION

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Few, if any, veterinarians actively engaged in the many facets of the profession are exempt from considering the effects of drug residues on their metabolites, particularly where food producing animals are involved. We must, I repeat, we must orient our thinking to always raise the question, "Does this use of this drug, antibiotic or pesticide result in residues that may possibly be toxic or cause to be unwholesome such products as meat, milk, and eggs?" Equally important is the question of the probabilities of such occurring.

Withdrawal statements based on knowledge of the drugs should be rigidly complied with to avoid such a probability.

The profession should have an excellent working knowledge of the withdrawal statements and should carry this message to the layman and be constantly on the alert for management and husbandry practices which appear to make such withdrawal statements impractical or unworkable. When such conditions are noted, he should feel morally bound to comment to the proper authorities for consideration of a solution.

In certain instances, under the prescription legend, the profession may have knowledge about drug residues not generally available to the public and only through this careful advice and direction to his clients will such withdrawal be obeyed.

As veterinarians we all are directly responsible for the health and welfare of the nation's animals. Equally important is our additional responsibility to the health and welfare of the ultimate consumer of the edible products from these animals. Veterinarians and other scientists in the Food and Drug Administration are one of the many groups involved in the first line of defense against unfit edible products. The Food and Drug Administration is involved when such products are made unfit through the use of drugs. The Administration insists that labeling spell out the areas of proper usage, dosage, administration and also that the labeling bear adequate withdrawal statements which are designed to preclude the possibility of occurrence of harmful amounts of drug residues or their metabolites in the edible products derived from animals. The professional user, namely the veterinarian, is the second line of defense in this respect, and should not create problems for veterinarians in government agencies responsible for the safety and wholesomeness of the food. The practitioner should not take the attitude that it is "Joe's job" in meat inspection to decide if any harm is done by injection of potent drugs into animals just prior to shipment. This will not be tolerated indefinitely by the public. Such an attitude creates a need for protection and results in legislation giving someone else the responsibility to do the job or creates severe restrictions for useful drugs.

*Veterinary Medical Officer, Division of Veterinary Medical Review, Bureau of Veterinary Medicine, Food and Drug Administration.
We must all, therefore, in all three areas—(1) the approver of the use of the drug, (2) the professional user, and (3) the inspector of the animal food—be firm, alert and cooperative in our effort to protect the ultimate consumer of the nation’s livestock.

Perhaps of particular interest to this group, or any professional investigator concerned with the safety and efficacy of drugs, are the regulations concerning veterinary investigational drugs. They were devised with the intent to protect the public from possible consumption of food containing harmful amounts of residues and the use of irrational or unsafe amounts on pet animals or livestock.

Scientists conducting research on drugs with animals should be thoroughly familiar with the context of this regulation. Particular attention should be given to the provisions for marketing edible products from these animals during or after completion of the test.

Protocol is also of particular interest to the scientists involved in field and clinical trials to prove safety and efficacy of a product. It would seem wise to submit for comment or discuss protocol with FDA prior to initiating large scale field or clinical trials. If the Food and Drug Administration considers the protocol deficient in certain respects, it is advantageous to know this in advance. If the protocol is actually deficient, the deficiencies should be corrected. If it is simply a matter of misunderstanding or difference of opinion, these can usually be resolved.

The next step in our first line of defense against harmful residues of drugs and their metabolites is the submission of the New Drug Application. Here we must have adequate knowledge and data on the amount and toxicity of the residue remaining in edible products prior to approval and for proper withdrawal statements (if necessary) concurrent with approval. Knowledge of usual husbandry practices in conjunction with residue data frequently gives us an indication of the probability of the occurrence of residue.

Beyond this step of approval of a drug for use on a food producing animal, veterinarians in Food and Drug are actively involved in Surveillance, Educational, and Regulatory Programs. These programs involve feed and drug industries, livestock owners, practicing State veterinarians, and Public Health and Department of Agriculture veterinarians. One of the main purposes of these programs is to prevent harmful residues from being present in edible products from animals.

Thus, it is not difficult to understand that drug residue is an important consideration for almost any veterinarian involved with livestock animals.
New awareness of the importance of proper drug usage by veterinarians and the livestock or poultry producer was stressed by the several speakers. Gabriel, discussing "Toxicology in Industry and Government," noted that toxicologists recognize the importance of drug safety for the producer, the animal and the public. Based on properly controlled experimental and clinical data, the indications, dosage, method of administration, possible toxicity, residue data and withholding recommendations can be made. The veterinarian, with his broad training in the basic medical sciences has contributed significantly to drug evaluation.

Two widely used group of drugs, the antibiotics and steroids, were reviewed by Gessert. Comparisons of their value, when used separately and in combination, were described. Again it was emphasized that proper diagnosis, along with knowledge as to the cause and course of the disease, was essential for the proper professional decision as to which drug or combination would be most efficacious. This would include an evaluation of the prior case history, and previous treatments which might jeopardize the animal's life if one of the antibiotics or steroids be given.

New and better analytical drug assay techniques have enabled those in the drug industry and government to more critically examine animal tissues for presence of residues. Today we no longer talk about parts per million (ppm.) but rather parts per billion (ppb.) or higher. This has enabled qualified experts, as Dr. Manly, of the Bureau of Veterinary Medicine FDA, to ask, "Does usage of this drug, antibiotic, or pesticide result in residues or their metabolites in amounts that may be toxic or cause to be unwholesome such products as meat, milk or eggs?" Withdrawal statements on all drug products should be rigidly complied with to avoid such a possibility.

In certain instances, under the prescription legend, the veterinarian has available potential residue data not known to the public. His careful advice and direction to his client will assure proper withdrawal of the drug prior to slaughter. FDA represents a first line of defense, utilizing the data provided by industry—the practicing veterinarian is the second line of defense to assure the public of wholesome, nutritious meat, milk and eggs.

The Committee, believing there is a great store of information on safety and efficacy of drugs not readily available to the veterinary profession for the benefit of animal and poultry health and thus mankind, and recognizing the probable serious urgency of increasing food production, recommends that the United States Livestock Sanitary Association, in conjunction with selected representatives from the Bureau of Veterinary
Medicine, the Council on Biologic and Therapeutic Agents of the American Veterinary Medical Association, and the drug industry take action to assure the early publication of a Compendium on all drugs of value to the animal and poultry industry and nation. This would include development of a plan of action leading to such publication with a regular review of new drugs and biologicals.

Information in such a Compendium would include, but would not be limited to such items as: Tradename; Generic Name; Description; Actions; Indications and Contraindications; Dosage; Administration; Warning Statements; Antidotes—both veterinary and human; and Withdrawal Recommendations. Where adequate data is not available, suitable guidelines and methods for securing such information would be developed. This would include encouragement and working with those in industry, universities or veterinary colleges or with other qualified investigators for securing data on drug safety, efficacy and freedom from toxicity.

Finally, the Committee strongly recommends that funds be secured to assure adequate scientific personnel for complete screening of all advertising media for scientific accuracy by the American Veterinary Medical Association prior to publication.

The papers presented by Gabriel, Gessert and Manly are presented as an addendum to this report.
INFECTIOUS BURSAL DISEASE (GUMBORO)

I. M. Moulthrop, D.V.M.*
Salisbury, Maryland

In 1962 Cosgrove observed a condition causing considerable mortality in young broilers and called it Gumboro Disease since it was very widespread in flocks near this little town in lower Delaware.

The same year Winterfield et al. isolated and identified a virus as the causative agent and suggested the term "infectious bursal agent." Helmboldt and Garner described the pathology of the disease in 1964.

The term infectious bursal disease (Gumboro) has been suggested by many and is now being used widely. It is a disease with rapid onset, rapid recovery and mortality ranging from 0.5 to 25 percent.

The onset is very rapid with birds appearing normal at night and finding many sick and dead the next morning, in most cases, under the hover. Sick birds sit on the sternum with the head down, often with the bill burrowed into the litter. The feathers are rough and frequently soiled at the vent with a stringy excrete which contains many urates. Some birds shudder at times while down and emit a loud protest when forced to move. Sick birds that are still moving about pick at their own vent indicating pain or itching.

The condition appears in young chicks from one and one-half to five weeks of age although it has been observed up to ten weeks. Recovered flocks seldom have many cull chicks left.

The nearly dead or dead chick has a very dry, rough skin. When the skin is pulled back from the breast, hemorrhages are observed on the inner thigh and, many times, over the breast muscle. The breast muscles are dehydrated enough to appear atrophied.

On opening the abdominal cavity, degenerated areas along the edge of the liver are present. These areas do not cover a very great portion of the liver area but are quite conspicuous. The bursa is greatly enlarged, very shiny and much edematous material gives the organ a yellowish glistening appearance. The edema seems to be on the outer wall of the bursa. Occasionally there is a cheesy core in the bursa. The bursa may be two to three times its normal size with much edema of the surrounding tissue.

The kidneys are pale and in some cases filled with urates so that one may see the outlines of the tubules.

The causative virus seems to be very stable. Several reports have appeared in trade journals pointing to this fact. There are also insect reservoirs, at least.

The lesser mealworm *Alphitobius diaperinus* (Panz.) was observed in many houses where the disease occurred. A number of these insects were collected from a house in which an outbreak had occurred eight weeks previously and placed in a clean glass jar with new shavings, fresh

*Maryland Livestock Sanitary Service Laboratory, Salisbury, Maryland.
chicken feed and held for one week. The worms were then ground in nutrient broth and a two ml dose of this suspension was administered orally to each of six, three-week-old susceptible chicks. These chicks were sacrificed on the fourth and fifth days postinoculation and typical lesions found upon necropsy. All birds were kept in isolation to prevent outside exposure. Uninoculated controls failed to reveal lesions at necropsy.

In controlling this condition, there are several approaches. One, of course, is good sanitation, disinfection and treating the house to remove any insect carriers that may exist.

Some farms have an initial outbreak with high mortality but subsequent flocks have little or no trouble. This type of farm is very prevalent and, of course, controls the condition itself.

Another group that I have designated the "problem farm" is one where flock after flock experiences greater than five percent mortality and resultant economic loss.

In an attempt to help owners of such flocks, our laboratory has developed an egg propagated virus that can be used on these "problem farms."

This egg adapted agent can be administered in the drinking water when the chicks are three to seven days old at the rate of four ml per one gallon per 1000 birds. These is evidence that the vaccine produces some very mild symptoms and there has been mortality up to two percent from all causes in the several hundred thousand birds exposed with our product. There is an uneventful recovery and the flock has excellent growth. The experimental flocks had much better weight and feed conversion at marketing time than did the controls.

In summary, there seem to be two avenues of handling this condition at this time:

1. No treatment for chicks on non-problem farms.
2. Expose chicks at an early age on problem farms.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

H. E. Goldstein, Columbus, Ohio, Chairman; R. I. Ammon, Trenton, New Jersey; R. A. Bankowski, Davis, California; F. G. Buzzell, Augusta, Maine; H. Aaberg, Chicago, Illinois; G. E. Coleman, Brunswick, Maine; D. P. Corbett, St. Louis, Missouri; L. C. Grumbles, College Station, Texas; J. E. Hanley, Dade City, Florida; R. Hogue, Lafayette, Indiana; A. E. Janwicz, Montpelier, Vermont; D. Koppenhofer, Deshler, Ohio; H. D. Mangus, Thornton, Indiana; S. A. Moore, Beltsville, Maryland; B. S. Pomeroy, St. Paul, Minnesota; P. Schroeder, Reedley, California; W. Schofield, St. Louis, Missouri; E. E. Stuart, Forest, Mississippi; J. W. Walker, Washington, D.C.; W. W. Worcester, Sacramento, California; T. Zweiart, College Station, North Carolina

The Transmissible Diseases of Poultry Committee has continued the effort of providing the stimulus for a national eradication program for Pullorum disease and Fowl Typhoid.

The subcommittee for the eradication of Pullorum disease and Fowl Typhoid continued its effort under the Chairmanship of Dr. A. E. Janwicz. This year's subcommittee membership was composed of six members of allied industry groups, four members of the United States Livestock Sanitary Association regulatory agencies and a member of the American Association of Avian Pathologists. The subcommittee held a meeting on May 24, 1966, in Washington, D.C. and submitted the following report:

The subcommittee recommended that a four phase program of suggested procedures be utilized as a model program to eradicate Pullorum Disease and Fowl Typhoid.

Phase 1 - Preparation

1. There should be a memorandum of agreement on the function of each responsible agency participating in the State Eradication program. That is, the official N.P.I.P. and N.T.I.P. office and the State regulatory official should have a written agreement on responsibilities. Blank memorandum of understanding should be provided to the States in order that the agreements could be developed in a reasonably uniform manner.

2. On national coordination there should be a memorandum of understanding of the function of the Animal Health Division and the Animal Husbandry Research Division.

3. The Extension Service in the States should be brought in for educational purposes.

4. The role of diagnostic laboratories and Accredited Veterinarians should be established in each program.

5. Participation in the N.P.I.P. - N.T.I.P. or its equivalent is suitable eligibility under Phase 1 (Section 3, A, (1), a).
Phase 2 - *Reduction of Incidence*

1. Pullorum disease and Fowl Typhoid should be reportable diseases. (Section 8)
2. There should be field follow-up of all isolations and outbreaks. (Section 17)
3. There should be proper procedures (quarantines) established for handling disposition of infected flocks and their products (Section 6)

Phase 3 - *Elimination of Outbreaks*

1. The program should be 100 percent voluntary participation of eligible flocks and hatcheries. The flocks and hatcheries could be participating in either N.P.I.P., N.T.I.P., or State program requirements.
2. There should be testing of all exhibitions and fancy birds (Section 2, F.)
3. States should institute minimum importation requirements for chicks and eggs (Section 12)

Phase 4 - *Protection Against Reinfection*

1. After the disease is declared eradicated in an area (State) the area should be designated as registered-free area (Section 4)
2. There should be provisions for instituting monitoring or surveillance testing program based on reduced testing. (Section 4, B, Section 5 B)

Phase 5 - *Total Eradication*

1. The disease could be declared in the total United States when all states reach Phase 4.

The Transmissible Diseases of Poultry Committee accepts and endorses the subcommittee report as written. The Committee recommends that the subcommittee continue for another year and suggests Dr. B. S. Pomeroy as Chairman.

The Committee further recommends that the 4-Phase Program for the eradication of Pullorum Disease and Fowl Typhoid be presented to the National Association of State Director of Agriculture group for consideration and possible endorsement.

The Committee recognizes and commends the National Poultry Plans Program changes in its attempt to up-date procedures providing closer uniformity with the United States Livestock Sanitary Association. Uniform Rules and Methods for the Eradication of Pullorum Disease and Fowl Typhoid.

This Committee recognizes the overall importance of Avian Tuberculosis in relation to the economic factors, the public health significance, and the relationship of total eradication. Your Committee studied the proposed Avian type tuberculosis eradication program providing for a Four Phase State-Federal Program. The Committee endorses this as a means of providing Uniform Rules and Methods for eradicating Avian Tuberculosis.
The Committee recognizes and commends the National Poultry Plans Proposal Programs for Mycoplasmosis control and recommends that the Executive Committee of the United States Livestock Sanitary Association support the program as printed in the Federal Registry.

The Committee recognizes and commends the Animal Health Division of the United States Department of Agriculture for its efforts in initiating a test pilot eradication program for mycoplasmosis, and encourages the coordination of this effort with existing programs.

Your Committee continues discouraging the use of planned exposure in control of mycoplasmosis. This program should be considered in the experimental stage. The Committee further recommends to the Veterinary Biologics Division of Agricultural Research Service, United States Department of Agriculture, that agency delay licensing Mycoplasma biologics until sufficient data can be provided to prove need in lieu of eradication.

The Transmissible Diseases of Poultry Committee notes with satisfaction that the Executive Committee of the United States Livestock Sanitary Association appointed a Salmonellosis Committee to cope with the professional aspect of Salmonellosis. This action originated from a resolution from the Transmissible Diseases of Poultry Committee. This Committee offers full cooperation with the Salmonellosis Committee.

Your Committee points out that Leukosis, Air saculitis, Synovitis, and others present great economic losses annually to our country's poultry industry. Research needs are many, in providing means for reducing these losses.

The Committee commends the Veterinary Biologics Division of the United States Department of Agriculture for its vigilance involving poultry biologics, and for taking concerned action this past year when poultry biologics were found contaminated.
ANIMAL TUMOR REGISTRY AS A SOURCE OF MORBIDITY INFORMATION

C. Richard Dorn, D.V.M., M.P.H.*

Fundamental to epidemiologic investigation is a knowledge of the incidence or morbidity of the disease under study. The most useful type of descriptive data is that based upon systematic collection of cases in a defined population. Regardless of whether the disease to be studied is parasitic, viral, nutritional, or of unknown etiology, the measurement of its natural occurrence and the characteristics of individuals that become ill are essential to understanding the etiologic process and developing means to prevent and control the disease. These principles apply equally well to diseases of livestock, pet animals, and humans.

Studies of the natural history of cancer in domestic animals have been hampered by inadequate data. There are few reliable sources of domestic animal cancer records, except for case series compiled at university veterinary clinics, usually under differing protocols and representing unknown portions of the total cases developing in the population. Therefore, new approaches to provide suitable information are necessary.

While tumor registries are not new, there are relatively few that collect domestic animal cases. The Armed Forces Institute of Pathology in Washington, D.C., under the sponsorship of the American Veterinary Medical Association, maintains the Registry of Veterinary Pathology which serves as a reference center of material for research and study of various types of animal tumors. In 1957, DeKock described the institution of a registry of canine neoplasms in South Africa.

Traditionally, tumor registries have been designed to collect cases for the study of the histopathological characteristics of various types of tumors as a basis for differential diagnosis. Some state human tumor registries, such as Connecticut, New York, and California, now emphasize the use of registry material for incidence rates as a measure of the morbidity of the disease in the population. Several morbidity surveys have been designed to collect similar data on leukemia in single animal species such as the canine study in New Jersey and bovine studies in California, Michigan, and Minnesota.

This report describes a general registry of histopathologically confirmed animal tumors of all types diagnosed in Alameda and Contra Costa Counties, California. A major purpose of the registry is to collect morbidity data by developing effective methods of systematic case collection in this defined area. In addition, the registry provides a source of morbidity data.

*From the Epizoology Section, California Cancer Field Research Program, California State Department of Public Health, 2151 Berkeley Way, Berkeley, California.

This study was supported by the U.S. Public Health Service Research Grant CA 05924, from the National Cancer Institute.
confirmed cases for analytical studies of effects of environmental factors and for comparative medicine studies of inter-relationships between cancer in different animal species and between cancer in animals and cancer in man. With a registry of confirmed cases, various followup methods can also be developed to learn more about the pathogenesis of the disease, effects of treatment, and survival.

ORGANIZATION

The registry was established in July 1963 within the California Cancer Field Research Program, California State Department of Public Health. Staff working with the registry include a veterinary pathologist, two veterinary epidemiologists, a biostatistician, a registry supervisor, two coders, and a consultant statistician.

A basic design consideration in this type of study is the choice of a suitable study area. Alameda County and Contra Costa County were chosen because they form a contiguous geographical and demographic unit, surrounded on two sides by water and on the other boundaries by areas of sparse human population. Current complementary human data are available for Alameda County through the Department's Human Population Laboratory and Alameda County Tumor Registry, a part of the California Tumor Registry.

Results of a preliminary survey of practices in this area indicated that few of the neoplasms clinically detected in the practices were confirmed by histopathology. Therefore, histopathologic examination of specimens had to be performed as a part of the study and the study area had to be confined to ensure a manageable volume of specimens. At the same time, the area had to be large enough to yield sufficient cases for statistical analyses. As considerable development of reporting procedures, field work, and mailing of specimens were required, an area close to the study office in Berkeley, Alameda County, California, was desirable.

Since it was to be a population-based study, an area for which population data were already available or could be collected was also essential. The comparable human and animal population data available for each county are shown in Table I. As the livestock populations are actually small for a tumor registry, it will take several years to collect enough cases in these species for analysis.

CASE REPORTING

At the beginning of the study, each veterinarian in the two counties was personally visited by a veterinary epidemiologist to explain the objectives of the registry and the reporting procedures. There were 40 practices and 54 practitioners in Alameda County and 25 practices and 34 practitioners in Contra Costa County at the beginning of the study in July 1963. Ten practices in surrounding counties and the Veterinary Clinic, University of California at Davis were also included as they occasionally diagnose neoplasm cases from the study area.
### TABLE I

Human and Animal Populations of Alameda and Contra Costa Counties, California

<table>
<thead>
<tr>
<th>Species</th>
<th>Alameda County</th>
<th>Contra Costa County</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>908,209</td>
<td>409,030</td>
</tr>
<tr>
<td>Dogs*</td>
<td>91,950</td>
<td>na</td>
</tr>
<tr>
<td>Cats*</td>
<td>73,096</td>
<td>na</td>
</tr>
<tr>
<td>Cattle</td>
<td>35,770</td>
<td>38,802</td>
</tr>
<tr>
<td>Sheep and lambs</td>
<td>26,463</td>
<td>10,018</td>
</tr>
<tr>
<td>Hogs</td>
<td>21,244</td>
<td>2,562</td>
</tr>
<tr>
<td>Horses and mules</td>
<td>1,131</td>
<td>1,340</td>
</tr>
</tbody>
</table>

na = Not available

*Owned dogs and cats

Sources:


Unpublished dog and cat population data collected in conjunction with Human Population Laboratory Survey, 1965, California State Department of Public Health.

The practitioners are asked to report their cases on the Neoplasm Case Report (NCR), shown in Figure 1, and to submit specimens for histopathologic examination. Preadressed, postage paid mailing containers for slide and tissue specimens are provided to each practice. In exchange for reporting a case, the histopathologic diagnosis is routinely returned to the veterinarian within 48 hours after the specimen is received. By this procedure, the diagnosis may be useful in the immediate care and treatment of the animal. Except for bovine lymphosarcoma which is a reportable disease in California to the State Department of Agriculture there is no requirement to report neoplasm cases. Therefore, the prompt return of a pathology report to the practitioner is thought to be a reasonable incentive.

Additional consultive services are available on individual cases and occasionally followup information is requested of the practitioner. Each practice is periodically visited by a veterinary epidemiologist who answers questions about the study and provides the mailing materials.

As each case is received, a quick reference file of the entire registry is searched for the owner's name, address, and animal's description, to determine if the animal has been previously reported. This system is capable of determining previous reports from the same practice or from different practices and has proven to be extremely valuable as the registry collects more cases. Currently, about 18 percent of the reports are from animals that have already been reported to the registry for either the same or different conditions. New animals are designated by an accession.
Figure 1. Neoplasm Case Report with IBM card column assignments identified with corresponding coding spaces.

Number; repeat reports from the same animal are designated by the registry number previously assigned to that animal. This permits assembling of all registry records on a given animal and avoids duplicate counting of animals that may have a number of specimens submitted from the same tumor.

Next, pertinent identifying information and the description of the site of the lesion are abstracted from the NCR and sent with the specimen to the pathology laboratory. The specimens, usually tissues from necropsy
or biopsy and blood and bone marrow smears, are processed using standard procedures and examined microscopically. Diagnoses are typed onto a pathology report form and copies are returned by mail to the practitioners.

DATA PROCESSING

All of the information from the NCR and the pathology report is assembled for each case and reviewed prior to coding. A Neoplasm Review Board, consisting of a veterinary pathologist, veterinary epidemiologist and representative of the statistical staff, decides registry procedures and coding policies. The Board also reviews certain cases such as those difficult to assign a primary site or histologic type category.

Occasionally, cases are reported for which no specimen can be procured. These case records are held in the registry but identified as clinical diagnoses and handled separately in the statistical analyses. The practitioners are encouraged to follow these cases in order to collect at a later time specimens at surgery, biopsy, or necropsy for histopathologic examination.

An IBM system is utilized for data processing. All of the information to be processed is converted into digital codes for keypunching onto IBM sorting cards. The codes are written on the NCR in the spaces shown in Figure 1, identified by their corresponding IBM card column assignments. This facilitates checking the codes against the original information and avoids the need for a transfer form, as the IBM sorting cards can be keypunched directly from the coded information on the NCR.

Detailed coding instructions have been developed for the items shown in Figure 1. The top part of the NCR collects identifying information and zoographic characteristics of the animal. The middle section of the NCR contains a group of questions asked of the owner. The first question is about parity which has been shown to be related to cancer risk in some species.\textsuperscript{14,15} Questions (11, 13, 14, 15) about usual residence, previous ownership, and where the animal is kept at the home, provide useful information for analyses of geographical distributions and associations with environmental factors. The American Kennel Club (AKC) question identifies a group of cases for which pedigrees can be procured for genetic study. The usual questions about when the condition was first noticed by the owner (onset) and when the case was first diagnosed by the veterinarian are asked, but only the latter is coded. The period of time between onset and diagnosis is calculated and coded. This interval may reflect both the speed of the disease progress and the owner's delay in bringing the animal for veterinary care.

Medical coding is accomplished from information abstracted from the clinical history on the NCR and the histopathologic findings. Several medical coding systems are used for versatility and comparability in analyses involving other animal and human cancer data. The Standard Nomenclature of Veterinary Diseases and Operations,\textsuperscript{16} developed by the Epizootiology Section of the National Cancer Institute, is used for coding
primary sites and histologic types of the neoplasms. Several schools of veterinary medicine use this nomenclature; many human hospitals and tumor registries use the Standard Nomenclature of Diseases and Operations, from which the veterinary nomenclature was adapted. The Standard Nomenclatures are very detailed, and the site categories are too numerous to facilitate statistical summaries. It is, however, possible to group together or collapse subcategories into major site headings using the Standard Nomenclature Site Groups developed by the California State Department of Public Health for the California Tumor Registry reports. This permits full use of the Standard Nomenclatures, while allowing the cases to be summarized for statistical reports. Another system used almost exclusively by the human tumor registries in their reports is the International Classification of Diseases. There is good agreement with veterinary terminology in the neoplasm section (140-239) and this system has proved to be indispensable in preparing statistical reports and in making comparisons with human cases.

In order to calculate incidence rates, new cases occurring in the population must be distinguished from old or previously diagnosed cases. Hence, the date a case is detected is critical for analyses. There are at least three dates that could be used: 1) the date first noticed by the owner (onset); 2) the date first clinically diagnosed by the veterinarian; and 3) the date the report is submitted to the registry. For the purpose of incidence analyses, the date first clinically diagnosed by the veterinarian (2) is thought to be the earliest reliable point to count it as a new case. Therefore, this date is used in the analysis of new cases occurring during a specific time period.

If computer equipment is used, the exact information desired is predetermined and proper instructions are programmed. A generalized statistical report program for the IBM 7090 computer that prepares cross tabulations (Figure 2), has been most serviceable in our data processing.

DESCRIPTION OF REGISTRY CASES

The results reported here are confined to a description of cases collected in the registry during the first two years of operation. Table II shows that the number of cases reported each year was nearly constant.

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<thead>
<tr>
<th>Date First Diagnosed</th>
<th>Date Reported</th>
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<tbody>
<tr>
<td></td>
<td>July 1963 - June 1964</td>
</tr>
<tr>
<td>All neoplasm cases</td>
<td>1,971</td>
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<tr>
<td>Prior to July 1963</td>
<td>284</td>
</tr>
<tr>
<td>July 1963 - June 1964</td>
<td>1,687</td>
</tr>
<tr>
<td>July 1964 - June 1965</td>
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TABLE II

Neoplasm Cases by Date of First Diagnosis Reported from Alameda and Contra Costa Counties, California, July 1963 - June 1965
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<tr>
<th>ISC CODE</th>
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<tr>
<td></td>
<td></td>
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<tr>
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<td>204</td>
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</tbody>
</table>

Figure 2. An example of a cross tabulation computer print out of registry cases. ISC codes are from the Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death, 7th Revision, 2 v., World Health Organization, Geneva (1957).

Sex codes: 1 = neutered female; 2 = entire female; 3 = neutered male; 4 = entire male.
TABLE III
Neoplasm Cases by Species Reported from Alameda and Contra Costa Counties, California, Initially Diagnosed July 1963 - June 1965

<table>
<thead>
<tr>
<th>Species</th>
<th>Date First Diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>July 1963 - June 1964</td>
</tr>
<tr>
<td>All neoplasm cases</td>
<td>1,847</td>
</tr>
<tr>
<td>Dogs</td>
<td>1,582</td>
</tr>
<tr>
<td>Cats</td>
<td>193</td>
</tr>
<tr>
<td>Pet birds</td>
<td>24</td>
</tr>
<tr>
<td>Pet rats</td>
<td>19</td>
</tr>
<tr>
<td>Horses</td>
<td>13</td>
</tr>
<tr>
<td>Cows</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
</tr>
</tbody>
</table>

The number of cases received during the first year, but first diagnosed in a previous period (284), was larger than the previously first diagnosed group (178) for the second year. This decrease may be due to a diminishing pool of undiagnosed cases in the population after the survey started.

Table III lists the number of animals of each species with newly diagnosed neoplasms during the two years. Most of the cases were dogs, followed by cats, and pet birds. Only a few cases in horses, cattle, and other livestock were received.

In the first year approximately 31 percent of the canine neoplasms were malignant, while 72 percent of the feline neoplasms were malignant. The charts in Figure 3 show the proportions of malignant neoplasms, by major sites, for dogs and cats. The most common sites in dogs were skin, mammary gland, hemic and lymphatic system, while in cats, the principal sites were skin, hemic and lymphatic system and mouth and pharynx.

Figure 3. Major sites of malignant neoplasms in dogs and cats.
Some interesting sex differences in the distribution of benign cases in dogs are shown in Figure 4. In comparison to all female benign neoplasm cases, there were proportionally more entire females than neutered females in the mammary gland tumor group. The perianal gland tumors were more common in males than in females. Conversely, the lipomas were more frequent in females, especially neutered females. The ratio of neutered to entire female lipoma cases was 3.3:1, as compared to the ratio for all benign cases of 1.4:1. This difference suggests that the development of lipomas may be associated with obesity which is sometimes observed in neutered females.

POPULATION AT RISK

In order to calculate incidence rates it was essential to have reliable estimates of the population at risk. Because all animals are not taken to a veterinarian when they develop a tumor, the population at risk for denominators of incidence rates using registry cases was smaller, only a portion of the total population.
To estimate the portion of the total population given veterinary attention, animal questions were included in a household survey of Alameda County conducted by the Human Population Laboratory. All of the pets in a household during the past five years were enumerated, and it was determined if any had been taken to a veterinarian. It was learned that 87 percent of the dog population and 75 percent of the cat population were from veterinary-using households. The data describing the animals enumerated in veterinary-using households were used for calculating population at risk estimates. Greater detail of the survey may be found in a separate report.

Using the Alameda County population at risk estimates and the Alameda County cases from the first year of the registry, the annual incidence rates for all types of malignant neoplasms were 423.0 per 100,000 dogs and 130.6 per 100,000 cats. If the total population were used, the rates would have been lower or underestimated. For example, using data compiled on the feline malignant lymphoma cases collected during the first two and one-half years in the registry, the total population estimates yielded an average annual rate of 31.2 cases per 100,000 cats, and the population at risk estimates, which takes into account veterinary use, yielded 41.6 cases per 100,000 cats. In addition, the age, sex, and breed distributions of animals from veterinary-using and non-veterinary-using households differ. Therefore, the total population estimates may obscure associations of these and other variables with a type of tumor or may result in erroneous evidence of associations.

DISCUSSION

It is unfortunate that morbidity studies in domestic animals are so limited by the nature of the data collected. For diseases that fluctuate temporarily in incidence by four- or five-fold or that are either present or absent in certain geographical area or species, conclusions based upon incomplete data may be correct. However, with chronic, ubiquitous diseases such as cancer and some of the infectious diseases, such as tuberculosis and brucellosis now reduced in incidence by control measures, the completeness of case-finding and appropriateness of the available population data are very important.

The problem of measuring the morbidity of animal diseases is not new. Several organizations, including the United States Livestock Sanitary Association, have promoted the improvement of animal disease statistics. A comprehensive historical review of animal morbidity and mortality reporting has been published by the Committee on Animal Health, National Academy of Sciences, National Research Council. The large number of attempts to improve completeness of reporting, quality, and comparability, documented in that report, is evidence of the enormity of the problem.

The registry described here represents a new approach to the problem of collecting useful animal morbidity data. In the study of both pet animal and livestock diseases there is similar need for diagnostic
confirmation, standard classification systems, and population denominators. It is hoped that some of the tumor registry procedures may be useful in other morbidity studies.

REFERENCES

The presence of *T. spiralis* in United States swine has long been a major problem from both the economic and the public health standpoint. Importation of United States pork products has been prohibited by many countries, partially because of the stigma that the possible presence of *T. spiralis* has produced. Only since 1963 has the market for pork expanded outside the United States; then only after the pork has undergone trichinoscopic inspection. The finding of the 16.1 percent incidence in the United States human population during 1937 to 1944 and the statement by Stoll\(^1\) that the United States has three times as many cases of trichinosis as the rest of the world combined, has helped to underline the public health aspects of the problem.

Zimmerman and Brandly\(^2\) found the rate of infection in 9,495 grain fed swine sampled during the period 1961-1965 to be 0.12 percent. This is less than one-tenth the rate (1.41 percent) found in an eight year survey of about eight million swine that was completed in 1906.

Studies to re-evaluate the prevalence of trichinosis in the human population of the United States are currently being carried out by the Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University with collaboration from the Communicable Disease Center, United States Public Health Service. The often quoted prevalence of 16.1 percent in humans was obtained in studies by the National Institutes of Health during 1937-44. However, since that time marked changes have been made in swine management and pork processing methods. The changes in swine management have contributed to a lessening incidence of the parasite in swine. The primary aim of the current human study is to determine the present prevalence of the disease in humans of the United States, thus determining if the changes in prevalence of the disease in swine is reflected by a similar decrease of the disease in the human population of the United States. The current study, with support from the National Institute of Allergy and Infectious Diseases, is a statistical designed study which will include approximately 10,000 human diaphragms from all 50 states and the District of Columbia. In addition to obtaining current prevalence, the study is also designed to determine possible relationships between prevalence and age, sex, residency, national extraction and other factors.

Through August 31, 1966, examinations have been made of 1093 human diaphragms, with 59 (5.4 percent) being infected. Infected samples have been obtained from six of eight states. A definite prevalence-age
relationship is indicated with the youngest infected cadaver being 41 years. Prevalence relationships are also tentatively indicated for certain nationalities, religions, and occupations. Only seven of the 59 infected diaphragms contained living trichinae.

The Interagency Committee on Radiological Assistance, composed of representatives of the Department of Defense, Office of Civil Defense, Coast Guard, Public Health Service, Department of Agriculture, Department of Interior and under the chairmanship of the Atomic Energy Commission met in 1966. Plans of the Committee for monitoring radiological activities were reviewed in detail.

Following a nuclear accident, and at the request of the Atomic Energy Commission, the participating agencies would commence monitoring activities in an area or nation-wide scale, depending on the need. Veterinarians now responsible for animal health and red meat and poultry inspection have been trained in radiobiology and in the use of monitoring instruments, which have been issued. These trained personnel and others such as members of the Division of Radiological Health of the Public Health Service, would, in the event of radiological contamination of the environment, assess the extent of the hazard. This will include monitoring contamination of milk, and other foods, animals, animal feeds, water and other items.

Refresher courses will be held regularly to maintain continued proficiency. Meetings to implement these plans are now being held at the Atomic Energy Commission regional offices throughout the United States.

REFERENCES

Salmonellosis effects more people and more animals than any other single disease. It is one of the most important public health-animal health problems. Salmonellosis may vary in severity from inapparent infections to acute disease, which may be fatal to the very young, the old, or the debilitated individual. It is estimated that there are two million persons infected each year in the United States. During the past quarter century reported salmonella infections in man in the United States, other than typhoid fever, have increased from 504 in 1942 to 20,867 bacteriologically proven infections in 1965.\textsuperscript{1,2} It is impossible to determine how much of the marked increase in reported human salmonellosis is due to actual increase in incidence of infections and how much is due to improved reporting. Methodology has improved during this period, but it is believed that wider application of known methods and more thorough epidemiological investigation of outbreaks have contributed most information about the occurrence and distribution of salmonellae.

As an indication of the magnitude of the reporting problem, during 1965 a waterborne salmonellosis outbreak occurred in Riverside, California; 200 cases were known to the Health Department. A door-to-door survey of the town increased this number to more than 16,000 cases.\textsuperscript{3} Thus, only slightly more than one percent of the cases in this outbreak would have been known had the original figures been accepted without investigation. The occurrence of salmonellosis in outbreaks involving multiple cases, whether among infants in nurseries, or extensive common source epidemics among adults, attracts attention and is widely publicized. However, it should be realized that these episodes represent epidemics arising from an endemic level of infection which is widely disseminated in the population.

The patterns of endemic human salmonellosis are becoming increasingly complex. The disease probably occurs in both sexes with equal frequency in all parts of the United States. The attack rate is highest in children under one year of age and then decreases rapidly, reaching a constant level in the 10-19-year age group. This level extends through the 40-49-year age group, after which a slight rise is noted, cumulating in a very modest second peak among people 80 years of age and over. These data depict salmonellosis as a disease primarily of youth, particularly of childhood and infancy. In contrast, approximately 70 percent of the reported fatalities associated with salmonellosis have occurred among those of 40 years of age and older. Most of the remaining 30 percent of reported fatalities have occurred in the infant population. The average reported case fatality ratio for each group is less than one percent.\textsuperscript{1}

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During 1963 and 1965 more than 50 percent of the salmonella isolations from man were reported as coming from sporadic cases, 20 percent from family incidents, 15 percent from general outbreaks and the remaining 10 percent from hospital-associated cases. Improved and more intensive investigations would no doubt result in a marked increase in those cases classified as sporadic incidents.

It is now well known that a certain percentage of persons who recover from acute infections, as well as individuals with a history of mild illness, may excrete the non-host adapted salmonellae for long periods. Only a small percent of these become permanent carriers as is seen with typhoid fever. Leader in 1956 and Szanton in 1957 documented the occurrence of long term, though not permanent, carrier states in children and the familial spread of infection through contact with convalescents. Domestic animals and many wild animals, wild birds, and reptiles may also carry salmonellae for long periods.

Reservoirs, Vehicles of Infection and Methods of Spread

Although salmonellae may inhabit most species of warm-blooded and many cold-blooded vertebrates, the major reservoir of human salmonellosis is in domestic livestock. The similar distribution of salmonella serotypes isolated from man and lower animals suggests that these organisms are transmissible from one to the other, or that they are derived from the same sources. Numerous studies demonstrate continuing infections among large numbers of domestic and wild animals, and it is reasonable to believe that continuation is due to a natural cycle in the individual hosts, or that there is a recycling mechanism from infected persons, animals, foods, or the environment. There is little to support the concept that salmonellosis in man is perpetrated by a closed human transmission mechanism. In contrast, paths of transmission from animals to man have been demonstrated many times. This evidence suggests that the main reservoir of salmonellae is in animals and that the organisms are transmitted to man either directly from animals or through contaminated products of animal origin. Transmission to man is secondary to the continuing cycle in animals.

More than two-thirds of approximately 25,000 cultures isolated from animals in the United States during the 30-year period from 1934 through 1964 were recovered from the domestic fowl. In the period 1963-65, during the first three full years of national salmonellae reporting to the Communicable Disease Center, 16,311 isolations were reported from nonhuman sources. Of these 12,287 were reported from four domestic species; turkeys 4,915 (40 percent), chickens 4,403 (36 percent), cattle 1,495 (12 percent), and swine 1,244 (10 percent). It is evident that domestic poultry is an important reservoir. However, from these reports, it is impossible to determine the relative prevalence of salmonellae in fowls and other animals, since fowls have been examined bacteriologically more often than other species.

The occurrence of salmonellae in the lymph glands of normal hogs and in retail pork products has been observed repeatedly. Studies of
the prevalence of salmonellae in asymptomatic hogs on farms, in holding pens, in sale yards, and in abattoirs clearly indicate that an increasing proportion of the animals become infected during transportation and while being held for slaughter.\textsuperscript{16} Compared with carcass pork, a much higher percent of processed meat products, such as sausage, have been found to be contaminated.

Reports now indicate that salmonellosis among dairy and beef cattle, particularly those held in close lots, is being recognized as a real problem.\textsuperscript{17-20} Clinically healthy cattle have been found to shed salmonellae for more than five months. Transmission of infection to man from infected cattle occurs primarily through consumption of raw or improperly pasteurized milk and milk products and from contaminated beef. Beef carcasses have been found to be grossly contaminated—probably reflecting poor handling. Recently, non-fat dry milk was epidemiologically in- criminated as the source of human salmonellosis; the same rare serotype found in the human cases was found in the dry milk used by the families.\textsuperscript{21-22} Further investigations have revealed a variety of salmonellae serotypes in this product.

In 1948, Edwards\textsuperscript{7} summarized data on 70 isolations from horses obtained during the preceding 16 years. More than half of these cultures were \textit{Salmonella abortus-equi} obtained from the genital tract of mares after abortion. The remaining cultures, with two exceptions, were \textit{Salmonella typhi-murium}. During 1965, 101 salmonella isolations were reported from equine sources; there were 13 different serotypes.

Salmonella infections are also prevalent in domestic pets, including dogs, cats, pet birds, and turtles. Various surveys have indicated that 15 to 20 percent of normal household dogs may be infected with salmonellae.\textsuperscript{23,24} The rate in dogs confined to kennels is usually much higher. Direct transmission of infection from dogs to man and vice versa has been observed.\textsuperscript{25} Pet chicks and ducklings, given to children at Easter, frequently are found to be the source of infection in the recipients.\textsuperscript{26} Parakeets in the home have been incriminated occasionally as a source of human infections.\textsuperscript{27}

Recently, a number of documented cases of salmonellosis associated with pet turtles were reported.\textsuperscript{28} More than 60 instances have been recorded in which the same salmonella serotype was recovered from the patient and the pet turtle involved. Studies in several states have indicated that a high percent of pet turtles for sale in retail stores are contaminated with salmonellae.

For years, rodents were thought to be among the most important reservoirs and transmitters of salmonella infection. Surveys of rat populations have indicated infection incidences from 0.7 to 13 percent and persistence of viable salmonellae in rat species for 148 days.\textsuperscript{29-31} The variety of serotypes isolated from rodents indicates that their salmonella flora reflects their environment. A few human infections have been reported as caused by contamination of food by rodents.

With the high rate of salmonella infections among animals, it is not surprising that many foods of animal origin frequently contain salmonellae.
Considerable evidence has accumulated concerning the presence of salmonellae in poultry meats, including partially frozen carcasses. Eggs and egg products have been the principal source of reported outbreaks of salmonellosis in man during the past three years. Studies of fresh pork sausage in retail markets have revealed as high as 35 percent of the samples examined contaminated with salmonella.

In addition to the long recognized problem in poultry, red meat, and egg products, recent reports have appeared concerning the presence and spread of salmonellae in such foods as soya milk, dried yeast, coconut, and cereal powder. Currently, carmine dye is being investigated as the source of Salmonella cubana infections. This dye is prepared from the cochineal insect and is imported from Peru and the Canary Islands. The investigations followed the isolation of S. cubana from a lot dye used as an intestinal marker in patients in a Massachusetts hospital. The dye is also found as a food coloring. It is interesting and perhaps significant that Brede reported the frequent isolation of Salmonella natal from cochineal bugs in South Africa in 1964.

Widely distributed animal feeds have been found to be heavily contaminated with salmonellae and provide an excellent means of spreading infections in domestic animals and fowl. Pets, domestic livestock, poultry, and laboratory animals have been involved in severe salmonellosis resulting from consumption of contaminated feeds. Various surveys of rendered animal by-products have revealed from 12 to 50 percent of the finished products were contaminated with salmonellae. Frozen horse meat has been found to carry many types of salmonellae. Fish meal is frequently contaminated with a variety of salmonella serotypes, derived usually from polluted waters and fish processing plants.

Vegetable foods used in animal feeds also have been found contaminated. For example, the same salmonella serotype has been found in cottonseed meals and in the organs of animals which consumed the product.

Whether salmonellae in animal feeds produces acute infections or only a carrier state, their presence establishes a path of transmission from feed through animals to man.

**Control**

The reported decrease in the incidence of Salmonella pullorum and Salmonella gallinarium infections in fowls from 70.4 percent in 1956 to 24.0 percent in 1963 illustrates the degree of control which can be attained in dealing with host-adapted salmonellae. Prompt application of testing procedures and use of disease free replacements have contributed to this decrease. Unfortunately, similar measures are not effective in dealing with the non-host adapted types because of their widespread distribution, as well as the many ways in which they may be transmitted. The rapid development of both human and animal food processing in this country, the widespread distribution of salmonellae in many of these foods, and the lack of bacteriologic standards to assure that human and animal foods and feeds are free from contamination has raised many problems. Epidemiological investigations of salmonellosis have repeatedly
demonstrated the importance of food and feed in spreading and maintaining the basic animal-to-animal and man-to-man cycles of infection. Nevertheless, the salmonella problem is vulnerable to control procedures that can be applied at certain points in the epidemiological chain of infection. When cases occur the sources of infection must be found, the chain of infection defined in each instance, and control measures formulated to prevent recurrence.

Regulations adopted during the last year by the United States Department of Agriculture requiring that all egg products processed under their inspection be pasteurized regardless of whether they are to be distributed in liquid, frozen, or dried form, constitute a first step towards the elimination of salmonellae from egg products and from foods containing eggs. During 1965 the Food and Drug Administration also passed regulations to require that egg products be treated to eliminate salmonella. The effectiveness of properly controlled pasteurization has been clearly demonstrated in the liquid milk industry. Similar regulations passed this year to require pasteurization of dry milk should be equally effective.

Control activities planned for the future must include continued and expanded surveillance of salmonellosis with a maximum of information exchange between state and federal agencies and the involved industries. Good surveillance is directly dependent upon good laboratory support. At the present time an insufficient number of laboratories are prepared to provide the necessary support for an intensified national salmonella control program; however, steps to correct this situation have been taken. The United States Department of Agriculture has established three regional laboratories, in addition to the main laboratory at Ames, Iowa, all of which will have competencies in salmonella bacteriology. Several industries now include salmonellae detection in their quality control bacteriology. Some industries have continuing research programs to study the sources of salmonella contamination in their products as well as methods of destruction of the organisms in the product. The Public Health Service has made plans to support the States in their salmonella control programs by providing special training courses in enteric bacteriology, by providing financial support to state laboratories, pilot control programs and, in some instances, by assigning Service personnel with special skills to the State programs.

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THE STATES' ROLE IN SALMONELLA CONTROL

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Someone has said that a state veterinarian determines the role his organization will play in any disease control program by three basic factors. These are:

1. Legal authority—both existing authority and new authority needed to act.
2. Personnel and funds available to him for the work.
3. The imagination and initiative that he possesses to bridge the gap between the first two.

Perhaps the control of salmonella differs from the other disease control programs we have tackled, but these differences are in areas that are familiar to us. It is the magnitude of the extension of these peculiarities of the salmonellae that overwhelms us. Here, we are dealing with a family of hardy agents (about 1,000 serotypes) that may infect all species of animal life and which may propagate without a living host.

The problems of adequate control measures for salmonellosis are not unique. I would like to quote from a speech that outlines very well the corrective steps needed. The authority for these comments is Dr. A. Granville, who is on the faculty of the School of Veterinary Medicine, Burssels, Belgium.

"In addition to the general and special hygiene necessary (to prevent the spread of salmonellae) in food production and utilization, the following measures can be suggested:

(a) "Compulsory declaration of cases of salmonellosis by physicians, veterinarians, and diagnostic laboratories.
(b) "The attention of the general public to be drawn to the ill-defined symptoms of enteritis that often reveal bacterial activity.
(c) "A tireless campaign to control forms of salmonellosis which are not well known—in other words, study the sources of infection and the human and animal carriers; break the man-animal-man cycle; try new methods of controlling the infection of food products (e.g., ionizing radiation).
(d) "Recommend that all perishable food be refrigerated and that all suspect foods (minced meat, food products based on eggs, milk, meals, etc.) be adequately cooked.
(e) "Establish effective cooperation between all public health (and agriculture) workers with a view to carrying out the most extensive possible epidemiological investigations. Do not be satisfied with
hasty results, which are often misleading. Improve diagnostic techniques as much as possible and raise the standards in specialized veterinary science."

I doubt if this list is complete, but it does give us an idea of what is required for a control program. Some of the measures fit into our sphere of responsibility; some do not. Yes, many different agencies are involved, each in a widely separated area of responsibility. Thus our role becomes more intricate, like the synchronizing of the mechanism of a watch where many parts are unknown to each other and yet all interdependent upon one another.

I am sure that there are many who are convinced that salmonellosis is not a significant disease in their areas, and perhaps many also who are even now unaware that comparable authority right next door may be quite involved in a Salmonella campaign. It appears that modern food production and distribution have not eliminated salmonella food poisoning and may have even increased the population at risk. When a contaminated food product enters the chain of modern food technology, outbreaks may occur in widely separated communities throughout the country. Public health workers believe that the animal population is the major source of salmonella for the human population. They believe that animals become infected most commonly through contaminated feed.

When we start thinking of Salmonella control measures, our past experiences will suggest much that can be put to use. A few well known tools merit attention. These are:

1. Authority to require reporting of all salmonella cases (human and animal).
2. Regulations that provide for inspection, testing of products, and instituting corrective measures in rendering plants and feed mills.
3. Authority to quarantine or prevent intrastate movement of infected animals, contaminated feed, and the like.
4. Authority to restrict importation of infected animals and contaminated feedstuffs.
5. Provisions for laboratory facilities for culturing specimens for the presence of salmonella.
6. An educational program aimed at improving sanitary practices on the farm and in the food and feed industries.

Most of us have some of these tools; but others may be a little hard to obtain, and we may have to improvise for a period. However, an inventory of the tools at hand and of the funds and manpower available can be easily made.

Perhaps the next step is to find where we fit into the salmonella control mechanisms that is developing.

A brief look at our teammates may help a little here.

1. For several years the public health services—both federal and state—have pursued the salmonella problem with enthusiasm and have even stimulated some public interest in this direction. It appears that
these people now consider food products of animal origin to be the main source of salmonella outbreaks in our human population. This has created some embarrassment for various agricultural industries. When food-stuffs such as powdered eggs and powdered milk are found to contain salmonellae, not only is there a loss to industry but a loss in consumer confidence in inspection services. We can expect the campaign against salmonellosis to continue, and we will be involved until animal agriculture is above suspicion.

2. During the forepart of this summer the Food and Drug Administration conducted a limited survey of salmonella contamination in rendering and fish meal plants throughout the United States. This may lead to a closer scrutiny of these products in interstate commerce. If seizures are made, we can certainly expect that the manufacturers of these products will be asking for consultation and assistance, including laboratory services, to help them solve their sanitation problems. A good hard look at our capabilities now, with some sound planning for the future, might pay big dividends in this general area.

3. All consumer and marketing service divisions that have food surveillance responsibilities have become more aware of the salmonella problem during the past year. They will be tightening their inspection procedures and production requirements on plants within their jurisdiction. This will undoubtedly be carried over into industries and plants under state surveillance and control.

4. For the past several years, the Animal Health Division has been studying the feed transmission cycle, and their future efforts probably will be primarily along the line of attempting to get industry and state support to prevent the manufacture and distribution of contaminated livestock and poultry feeds. However, there are other modes of transmitting salmonella, and the Animal Health Division will soon be looking at other factors which prevent the arrival of livestock and poultry or their products in the market place free of salmonella. Usually our role with the Animal Health Division is one of 50-50 cooperation, but the state veterinarian generally works directly with members of industry within his state.

5. There are many other state and federal agencies with varying degrees of responsibility in this problem, and we can surely expect to see some degree of association with each of them developing in the future.

It appears that the role of the state veterinarian in the salmonella problem will be an ever-increasing one. Perhaps, even now, we should at least begin to think in terms of an effective official control program.

1. As a beginning, we might review our legal authority to deal with the salmonellae and, where necessary, start the action that can obtain just the tools we need.

2. Our diagnostic laboratories, as stated, may be called on to culture samples and/or specimens for salmonellae in excess of past years' workload. In some cases, this may require additional funds and personnel, and it certainly is not too soon to begin our planning for this increase.

3. The Animal Health Division will be asking to continue random
feed-product monitoring and culturing and, in some states, seeking a voluntary cooperative control program with renderers and, later, feed mills. We will need to establish sound working relationships with these industries in our respective states.

4. We need to improve our liaison with the Public Health Service, the Food and Drug Administration, the Bureau of Commercial Fisheries, and local medical groups. Most importantly, we need to make our efforts known to them and to learn of their activities.

I would like to summarize by saying that we should all recognize that the salmonellae in animals, humans, foods, and feeds represent a complicated picture or problem, as the case may be, and it is in many instances an interlinking one. The salmonella problem is not one that will go away if we try to ignore it. In our ever-expanding and increasingly complex society, with the attendant increasing emphasis on the production of concentrated animal protein, the attention given to the salmonellae and the concern of responsible agricultural and public health professionals about these microorganisms will undoubtedly be increasing.

It is not an unsolvable problem. As animal health scientists, we have faced and overcome problems as complex as this one in the past. We have actually made responsible and effective beginnings with less information than we now possess about the salmonellae. Increased surveillance across the board, now, is a start and it is a must. We have so far identified a thousand or more serotypes since the first isolation from swine by Salmon and Smith in 1889, following what was perhaps the original by Moore from a pigeon in 1885. Our present thinking is that only a small fraction of these serotypes represent a specific pathogenic threat. This fact in itself may have led many to feel that emphasis is being placed on the salmonellae out of all proportion to their importance. If there were some truth in this belief, and I am not at this point ready even to suggest it, ever-increasing surveillance of foodstuffs and feedstuffs would still be justified. Is it just possible that what we see emerging here is a reliable and perhaps consistently accurate measure of the degree of efficiency with which acceptable practices and procedures are applied to the production and processing of our foodstuffs and feedstuffs? May not surveillance for the salmonellae in the food and feed industries serve the same useful purpose as continual surveillance for the coliform organism serves in the dairy industry? If this be true, then when the warning flag goes up, it is our responsibility as state veterinarians to heed it and to do something about it.

REFERENCE

REPORT OF THE COMMITTEE ON SALMONELLOSIS

T. J. Gremnan, Jr., Providence, Rhode Island, *Chairman;* C. E. Boyd, Columbia, South Carolina; J. R. Hay, Western Springs, Illinois; J. G. Miller, Tifton, Georgia; R. E. Omohundro, Hyattsville, Maryland; J. H. Steele, Atlanta, Georgia

For many years, persons in responsible positions, including livestock health officials, turned their backs on the potential of salmonellae. It was looked upon as a sometime project, but nothing to cause concern.

As more and more information has become available, it is apparent that far from being a project, the presence of salmonellae is very definitely an important factor in our over-all environmental pollution.

The problem is of tremendous magnitude. It is not one that cannot be overcome.

The Animal Health Division, United States Department of Agriculture, is concluding a large-scale feed sampling survey, to determine the extent of salmonellae contamination of livestock feeds and feedstuffs.

Twenty-six states were included in the sampling area.

Preliminary results of this survey were presented to this Committee. At this stage, however, no determinations nor final interpretations can be made (seventy percent completed). The survey has shown that the production of salmonellae-free animal by-products must be effected in order to reduce the transmission through finished feeds.

The first effort in this direction is the proposed federal-state cooperative Salmonella Program as outlined below.

*Project No. 1—Inspection and product testing in rendering plants.*

This program will be offered in as many of the 26 States in the testing area as funds permit.

A State or Federal field veterinarian will visit each animal and poultry rendering plant to offer the plant management the opportunity to participate in a voluntary rendering plant evaluation and testing program. If a plant agrees to participate in the program, the veterinarian will conduct an inspection tour of the plant with a member of the plant management noting obvious lapses in sound sanitation practices. Sanitation procedures outlined in *ARS 91-47 (Recommended Sanitation Guidelines for Processors of Poultry and Animal Byproducts)* should be used as a guide.

A report of inspection listing obvious sanitation deficiencies and corrective actions recommended should be given to the plant management. Additional copies of the inspection report will also be needed.

Five randomly selected samples of finished product should be collected as outlined in *ARS 91-47*.

Each plant should be inspected and tested at least once during FY 1967 but preferably each six months to the extent that funds are available.

Plants with positive tests should be revisited by the inspecting veterinarian and an intensive examination be conducted of the operation with
REPORT OF COMMITTEE

the appropriate plant personnel in an effort to determine where sanitation is breaking down. It is expected that additional finished product and environmental swab samples should be collected and tested to help determine the source of the recontamination problem. To the extent that funds are available, field personnel should continue to work with problem plants until they are producing a salmonella-free product.

In December, 1965 the American Veterinary Medical Association conducted a survey of the states regarding laws, enforcement agencies, inspection and sampling procedures. Forty-seven states responded.

Of these forty-seven, forty-four states indicated a total of 674 rendering plants.

Twenty-five states indicated state laws.
Twenty-two states indicated no laws.
Thirty-one states inspect rendering plants
Fifteen states do not inspect.
The frequency of inspection varies from once annually to six times annually.
Thirteen states take samples for bacteriological examination.
Thirty states do not sample.

Many existing state laws are funding in nature, but do not provide for adequate enforcement.

One of the initial aims of this Committee is to develop a uniform model law concerning the control of salmonella to be adopted by the individual states, of so recommended by this Association. Adequate enforcement provisions are needed to fully supplement the federal-state program outlined above.

We point out the need for developing a central agency to collect, coordinate, and to make available, information presently being compiled by various agencies.

To urge all agencies involved, and in particular the Animal Health Division, United States Public Health Service, Food and Drug Administration, to increase their efforts to provide training for state personnel in the area of detection methods.

There is a definite need that a symposium be developed. We recommend that feed manufacturers, fabricators, and distributors; animal breeders, and producers; meat packers and processors; supporting laboratories, and agricultural and industry laboratories be included.

This Committee has noted current increases in federal appropriations, but emphasize that they fall far short of the needs of the various agencies. We urge the Federal State Relations Committee of this Association to fully support appropriation requests of the agencies involved, and we ask the membership of this Association to join in this effort.
SHEEP FOOT ROT CONTROL

George L. Crenshaw and Blaine McGowan*

Davis, California

At the request of the California Wool Growers Association and the Sheep Committee of the California Farm Bureau Federation, a sheep foot rot control pilot program has been initiated in California.

This program is being conducted through the cooperative efforts of the following groups or agencies: California Bureau of Animal Health; California State Department of Agriculture, Bureau of Animal Industry; California Veterinary Medical Association; the University of California School of Veterinary Medicine and the Agricultural Extension Service; and the California Wool Growers Association.

The program consists of two parts: 1) Field research and 2) laboratory investigations. At the present time only field research is being conducted consisting of intensive investigations in four cooperating bands of sheep in northern California.

Work on all bands was started in May and June 1966. All sheep have been inspected at monthly intervals with the exception of one band. In the latter group inspection has taken so much time they have only been completely examined twice.

The procedure has been as follows:

1. Trim and examine each foot of all sheep on the ranch.
2. Segregate all affected animals into either:
   a. Treatment group
   b. Cull group
3. Put all clean sheep through a saturated bluestone or five percent formalin foot bath and place in a field which has been unused for at least two weeks.
4. Treatment group held close to corrals for consistent treatment.
5. Eliminate, where feasible, all cull animals as soon as possible.
6. Re-inspect treatment group in approximately two weeks. Cull or re-treat as indicated by response.
7. Re-inspect presumed clean ewes adhering as closely as possible to a 30-day schedule.

A procedural outline which has been our guide in this program is as follows:

FOOT ROT CONTROL FIELD PROGRAM

CONTROL METHODS

1. Trim all feet on every animal and dip.
   Treat affected and isolate.

*School of Veterinary Medicine, University of California, Davis California.
Affected animals identified by numbered ear tags and brands.

Treatment:
- Identification by color brand.
- Use bottle brand over affected legs.

2. Re-treat affected animals a minimum of every three days with inspection at time of fourth treatment. Retrim as necessary.

3. Recovered sheep go to a separate group.

4. After two clean inspections and foot baths 30 days apart, they may go to a clean band.
   Infected group may be re-treated for another series as described in "2".

5. Clean band:
   - Must pass two clean inspections 30 days apart.
   - Flock must be inspected and trimmed at least twice yearly.
     One inspection to be approximately three weeks after the first heavy rain.

6. Introduction of new sheep—rams or ewes:
   Must pass two clean inspections and be trimmed 30 days apart.

MEDICATIONS

- Bluestone ............. saturated solution (4#/gal.)
- Formalin ............. 10 percent applied topically
  five percent foot bath
- Chloromycetin ........ 10 percent solution in alcohol applied with a brush
- Kopertox ............. applied topically.

TREATMENT

1. Individual:
   - Formalin—10 percent
   - Chloromycetin—10 percent
   - Kopertox

2. Group: Foot bath
   - Bluestone, Saturated
   - Formalin—five percent

PREVENTION

Foot bath:
- Bluestone (saturated solution)
- Formalin—five percent

Results to date on the disposition of sheep as to foot rot status has been compiled on three of the four bands of sheep and are as follows:
**SHEEP FOOT ROT CONTROL**

**RANCH 1**

*May 24 and 25*

- 1017 ewes and rams
  - 93 to treatment group
  - 91 culls
- Balance: 833 clean ewes and rams
  - 93 in treatment group

*June 22 and 23*

- 833 clean ewes and rams
  - 9 more to treatment group
  - 93 in treatment group
  - 10 culls
- Balance: 824 clean ewes and rams
  - 92 in treatment group

*July 18 to 21*

- 824 clean ewes and rams
  - 27 more ewes to treatment group
  - 5 more rams to treatment group
  - 3 culls
- 500 yearling ewe lambs
  - 8 to treatment group
- Balance: 789 clean ewes and rams
  - 492 yearling ewes
  - 132 in treatment group

*August 24 and 25*

- 789 clean ewes and rams
  - 5 more to treatment group
- 492 yearling ewe lambs
  - 0 to treatment group
- 132 in treatment group (Sept. 6)
  - 0 culls (Found five with slight foot rot, but these remained in treatment group)
  - 3 dead (from clean band; cause of death undetermined)
- Balance: 781 clean ewes and rams
  - 137 in treatment group
  - 492 yearling ewe lambs

*September 21-23*

- 781 clean ewes and rams
  - 2 to treatment group
- 492 yearling ewes
  - 0 to treatment group
- Balance: 779 clean ewes and rams
  - 492 yearling ewes
  - 139 in treatment group
Incidence of foot rot:
Older sheep ....................... 23.0 percent
Yearlings ......................... 1.6 percent
Older sheep plus yearlings ....... 15.8 percent

RANCH 2

May 14-21
1219 ewes, rams (including 396 yearlings)
  100 to treatment
  75 culls

June 20-21
1044 clean ewes, rams, yearlings
  10 to treatment group
  4 culls
Balance: 1030 clean ewes, rams, yearlings
  110 in treatment group

July 20-22
1030 clean ewes, rams, yearlings
  0 to treatment group
  10 culls
Balance: 1030 clean ewes, rams, yearlings
  100 in treatment group

August 29-31
1030 clean ewes, rams, yearlings
  1 to treatment group (ram)
  100 in treatment group
  0 culls
Balance: 1029 clean rams, ewes, yearlings
  101 in treatment group

Incidence of foot rot:
Older ewes plus rams plus yearlings . . . . 15 percent

RANCH 3

June 16
1464 ewes and rams
  277 to treatment group
  230 culls
  231 yearling ewe lambs
  4 to treatment group
Balance: 957 clean ewes and rams
  227 clean yearlings
  281 in treatment group
August 15
975 clean ewes and rams
16 to treatment group
8 culls
281 treatment group
29 culls
Balance: 933 clean ewes and rams
268 in treatment group

September 20
277 yearlings
2 to treatment group
Balance: 225 yearlings
270 in treatment group

Incidence of foot rot:
Older sheep ................. 36.0 percent
Yearlings .................. 2.6 percent
Older sheep plus yearlings . . . . 31.7 percent

As can be observed, the incidence of sheep manifesting foot rot is 20 percent or higher in ewes. On Ranch 2, the over-all incidence is lower because the yearlings and aged ewes were run together. When this ranch is compared with Ranch 1's over-all incidence, they are practically the same, i.e., 15.6 percent vs. 15.8 percent.

It is also obvious that a low percentage of yearling ewes are infected, with a low of 1.6 percent and a high of 2.6 percent.

Other observations made to date are:

1) Treatment may not be required if proper trimming methods are used and sheep are on dry pastures.
2) There is a great deal of variation among trimmers. A well-trained crew is extremely important. It is easier to train a crew unfamiliar with foot rot, because they have no preconceived opinions.
3) It is preferable to trim early in the day and stop when trimmers get tired. Fatigued personnel often let infected sheep get into a clean band.
4) When isolation is difficult and facilities limited, sheep with a poor prognosis should be culled when inspected.

When a full season has been completed it should be possible to make a more comprehensive report. Foot rot in sheep is much more prevalent during the rainy season which occurs in California during the late fall, winter, and spring. Any evaluation of this program would be premature without a complete cycle of seasonal experiences.
RAM EPIDIDYMITIS VACCINATION*
G. L. Crenshaw and Blaine McGowan
Davis, California

INTRODUCTION

Epididymitis in rams caused by the ram epididymitis organism (REO) has been recognized as a major sheep disease in California for the past decade.

The incidence in rams in commercial flocks varies, but it has been high enough in most flocks to be of significance. McGowan and Shultz examined 1,882 rams and found 491 or 27 percent manifested clinical evidence of the disease.

Serological examinations of clinically negative rams using the CF test revealed 13 percent of 1,000 rams positive in one survey while in another study 23 percent of 400 were found to be serologically positive.

Semen evaluations conducted on clinically evident infected rams revealed that only 25 percent produced strongly fertile semen.

The objective of this study was to determine the effectiveness of the REO vaccination for control of ram epididymitis under field conditions.

MATERIALS AND METHODS

Four commercial breeders' rams were used in this study. Three of the trials were initiated in 1962, whereas the fourth was started in 1963.

On the first three ranches during 1962 and 1963 only an alum precipitated REO bacterin prepared at the School of Veterinary Medicine, University of California, was administered simultaneously with Brucella abortus Strain 19. Subsequently, only REO bacterin was administered to rams in these flocks. On the fourth ranch only REO bacterin was administered.

When REO bacterin and B. abortus Strain 19 were used, only a single injection of each was administered simultaneously, whereas when REO bacterin was used alone, two injections were administered approximately 30-60 days apart.

Initially in each group all rams, clinically free of epididymitis, were vaccinated. Also, all newly introduced yearling rams were vaccinated prior to being used for breeding.

Serological tests were conducted on each of the lesion-free rams. Since a high percentage of these rams, 25-36 percent, were positive, it was considered economically impractical to eliminate these rams, and all rams regardless of the serological results were vaccinated.

In 1964 a commercially prepared REO bacterin** was made available. As of that year all rams were vaccinated with this bacterin.

*School of Veterinary Medicine, University of California.
**Ramedol—Cutter Laboratories.
Until 1965 all yearling rams were vaccinated on the ranches. Vaccination of rams for sale at most ram sales was initiated, and unless the owner purchased unvaccinated rams, no bacterin was administered to these newly purchased animals.

All older rams in the flock were examined annually and concommitantly received a booster REO vaccination. Examination by palpation and revaccination usually was done in the spring. Initially, rams with unilateral or minor involvements were left in flocks 1, 2, and 3. Subsequently, in 1963 and thereafter any rams with clinical epididymitis were culled.

The REO bacterin was administered subcutaneously, posterior to the point of the olecranon. Dosage consisted of five cubic centimeter each injection. B. abortus Strain 19, when used, was administered in a similar manner.

Rams on Ranch 4 were more rigidly inspected and culled than on the other ranches. They were examined three times each prior to and following vaccination.

In 1966 testicles from rams and Ranches 1 and 3 manifesting lesions were cultured for REO and psittacoid (PLV) virus.

RESULTS AND DISCUSSION

Vaccination with REO causes irritation and soreness for a period of five to ten days. Frequently granulomas may occur, and rarely abscessation results from vaccination. None of these factors, however, have proved to be a problem in any of the ram studs used in these trials.

**TABLE I**

Incidence of Rams with Epididymitis at Ranch 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Rams</th>
<th>Number with Lesions</th>
<th>Percent Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>60</td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>1963</td>
<td>50</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>1964</td>
<td>52</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>1965</td>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1966</td>
<td>45</td>
<td>7*</td>
<td>16</td>
</tr>
</tbody>
</table>

*All testicles cultured. REO recovered from 2 rams, or 4.4 percent.

The incidence of ram epididymitis on Ranch 1 (Table I) was reduced gradually and significantly through 1965 when no clinical cases were observed. In 1966, however, seven of 45 rams manifested epididymitis, or lesions similar to epididymitis. During the 1965 breeding season, one ram died from an acute orchitis. Another was severely affected, and the testicles were obtained for examination. A PLV agent was revealed to be the causative agent.

Following examination of this group of rams in the spring of 1966, all affected animals were sent to slaughter and the testicles obtained for culturing. REO was recovered from two of seven rams.
TABLE II
Incidence of Rams with Epididymitis at Ranch 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Rams</th>
<th>Number with Lesions</th>
<th>Percent Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>25</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>1963</td>
<td>26</td>
<td>2</td>
<td>7.7</td>
</tr>
<tr>
<td>1964</td>
<td>25</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>1965</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1966</td>
<td>25</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

On Ranch 2 (Table II) the incidence of epididymitis was low initially, was reduced significantly the first three years, and remained minimal in 1966.

TABLE III
Incidence of Rams with Epididymitis at Ranch 3

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Rams</th>
<th>Number with Lesions</th>
<th>Percent Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962</td>
<td>15</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>1963</td>
<td>12</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>1964</td>
<td>12</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>1965</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1966</td>
<td>19</td>
<td>4*</td>
<td>21</td>
</tr>
</tbody>
</table>

*All testicles cultured. REO recovered from one ram, or 5.3 percent.

Rams from Ranch 3 (Table III) were more severely infected and the incidence was not reduced until 1964. No rams with lesions were observed in 1965. In 1966, however, four of 19 rams evidenced lesions when palpated. All testicles from affected rams were obtained and cultured. REO was recovered from one ram.

TABLE IV
Incidence of Rams with Epididymitis at Ranch 4

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Rams</th>
<th>Number with Lesions</th>
<th>Percent Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963-64</td>
<td>66</td>
<td>37</td>
<td>56</td>
</tr>
<tr>
<td>1964-65</td>
<td>65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1965-66</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1966-67</td>
<td>123</td>
<td>2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Ranch 4 (Table IV) had an extremely high incidence of epididymitis. The results were spectacular, but probably attributable to the vigorous examination program used initially. Additionally, a rigid booster
vaccination program has been maintained with even the replacement rams receiving vaccine. It is doubtful that such excellent results will be maintained, but rather that the pattern will be similar to the first three ram groups.

In general, when the results of vaccination are compared with non-vaccination and culling of rams with lesions, the difference has been significant. When the latter method is used, the incidence of palpable lesions remains at approximately 27 percent as previously described¹ which is higher than any incidence recorded in the ram studs included in our study. This result has also been reported by practicing veterinarians.³

Epididymitis vaccination programs and close observation of vaccinates have obviated the possibilities of other organisms causing epididymitis and orchitis.

The vaccination program as designed has been well accepted by commercial sheepmen and is one which they consider highly desirable to continue.

Some of the significant effects of this program have been:

1. More lambs are dropped earlier in the season resulting in less "tail-end" lambs.
2. With the reduction of epididymitis, owners are able to cull more for other defects.
3. Sheepmen are taking more interest in their rams as reflected by:
   a. Better conditioning of rams
   b. Increased cognizance of the breeding activity of individual rams.

SUMMARY

Ram epididymitis caused by the ram epididymitis organism (REO) is a problem of major importance in California.

The incidence of this disease has been significantly reduced by vaccination with a bacterin.

No marked adverse effects have been observed due to vaccination.

Other agents have been incriminated as causative factors of epididymitis and orchitis.

The vaccination program has been well accepted by commercial sheep producers.

REFERENCES

3. Dickson, Robert and Ned Haley.: Personal communications.
4. Unpublished information on other causative agents related to epididymitis or orchitis.
REPORT OF THE COMMITTEE ON DISEASES
OF SHEEP AND GOATS

L. E. Bodenweiser, Albuquerque, New Mexico, Chairman; P. C. Bennett, Ames, Iowa; W. J. Hadlow, Hamilton, Montana; J. L. Hourrigan, Hyattsville, Maryland; A. K. Kuttler, Boise, Idaho; B. McGowan, Davis, California; R. I. Port, Cheyenne, Wyoming; R. E. Simmons, Boise, Idaho; O. H. Timm, Dixon, California; W. Van Horn, Buffalo, South Dakota; H. Versluis, Salt Lake City, Utah; U. P. White, III, Roswell, New Mexico

Mr. Chairman, Members of the United States Livestock Sanitary Association: Your Committee reviewed several diseases and current problems pertaining to sheep and goats. Among these were sarcosporidiosis, with particular reference to sheep; scrapie; foot rot; and ram epididymitis.

A detailed report on sarcosporidiosis and a short summary on the present status of the scrapie eradication program is being submitted to the Executive Committee for inclusion in the proceedings for this meeting.

You have just heard presentations on ram epididymitis and foot rot, including a film on the latter. Your Committee recommends the inclusion of these presentations in the proceedings.

The Committee commends and expresses appreciation for the excellent report on sarcosporidiosis by Dr. A. M. Carey of the Livestock Slaughter and Inspection Division, Consumer and Marketing Service, United States Department of Agriculture, and recommends that the Consumer and Marketing Service continue their study of the incidence and economic loss caused by this condition and that the states concerned make an effort to trace back sheep found to have the disease at slaughter and to ascertain the possible source of infection.

The Committee directs the Association's attention to our recommendations of last year on foot rot and epididymitis and suggests that regulatory officials give strong consideration to these recommendations.

Respectfully submitted for your approval and reference to the Executive Committee.

SCRAPIE OUTBREAKS DURING FISCAL YEAR 1966

Scrapie was reported in nine flocks in four States during Fiscal Year 1966, compared to 12 outbreaks the previous year. All outbreaks during 1966 involved Suffolk sheep. The number of flocks under surveillance for scrapie has dropped to 518, the lowest since the beginning of the Scrapie Eradication Program in 1952.

The three outbreaks occurring in Illinois, two in Hancock County and one in Effingham County, were reported by a veterinary practitioner. The two outbreaks, in Hancock County, had the same source within that county.
The third, in Effingham County, involved a sheep born in a Shelby County flock. Both source flocks had been dispersed. The infected and source flocks and bloodline sheep moved from these flocks were slaughtered or taken to Mission, Texas, for use in the Field Trial Study. Nonbloodline animals were placed under surveillance.

The four infected flocks in Texas were in Tarrant, Collin, and Denton Counties. Three of the outbreaks were disclosed by inspectors at the Fort Worth Public Stockyards and the fourth was reported by the owner. Three of the four flocks are believed to have had a common source in Grayson County. The source of the other flock was not determined. The infected and source flocks were slaughtered as were exposed sales from these flocks and their immediate progeny.

An outbreak was disclosed in Colorado for the first time by a veterinary practitioner in Kiowa County. The source is believed to be a Stanton County, Kansas flock. Disposition of the flocks involved is being determined.

The policy followed in Texas and Illinois was to slaughter all infected flocks and all source flocks still in existence in their entirety.

FURTHER INFORMATION ON FISCAL YEAR 1965 OUTBREAK

In Fiscal Year 1965 an attempt had been made to remove bloodline animals only from an Illinois infected flock and its source flock. The bloodline animals from these flocks were purchased for use in the Mission Field Trial Study. At the time of purchase a second ewe (half-sib to the affected ewe) was showing early signs of scrapie and was held in Illinois. The diagnosis was subsequently confirmed histopathologically. Later at Mission, Texas, two bloodline sheep, one from the infected flock and one from the source flock, were confirmed cases also. Furthermore, in the source flock another ewe showed "scrapie-suspicious" signs; however, suitable tissue for histopathological study was not obtained. These additional cases involved more than the original bloodlines. Subsequent to diagnosis of these additional cases the remainder of the infected and source flocks were slaughtered or taken to Mission.

SCRAPIE FIELD TRIAL

The Scrapie Field Trial at Mission, Texas, is designed to hold different categories of sheep or goats under close observation for an extended period of time to learn which animal will develop scrapie and thus provide information which should assist in program direction.

There are presently 13 bloodline, but not exposed, sheep on scrapie-free premises No. 1. These include the natural increase from seven half-sibs, via a ram, to affected sheep taken to Mission in April 1965. There has been no evidence of scrapie in this group to date.

The majority of the animals at Mission are on scrapie-infected premises No. 3. This includes 190 "bloodline" sheep of one or more of the following categories: progeny of affected rams or ewes; dams or sires of
affected sheep; half or full-sibs to affected sheep; grandprogeny of affected sheep; grandprogeny of sires or dams of affected sheep; ewes mated to the sire of affected sheep, and scrapie suspicious sheep.

In order to evaluate contact transmission under field conditions, 99 nonbloodline previously nonexposed sheep and 17 goats of the same category were purchased and placed in contact with affected and/or bloodline sheep on the infected premises. None of these sheep consisting of 30 ewes and three rams for each of the Hampshire, Rambouillet, and Targhee breeds or the 17 goats of Angora, Nubian, and Toggenburg breeds have been subject to exposure for more than 15 months.

Since November 1964 a total of 16 sheep at Mission have died or been destroyed because of scrapie. Six were suspects held for observation, four were full-sibs to affected sheep, four were half-sibs to affected sheep, via the sire, and two were progeny of affected ewes.

While the Field Trial has not been underway a sufficient length of time to draw any firm conclusions, some preliminary observations may be of interest.

There are some indications that the histopathology of scrapie sheep from one section of the country differed somewhat from the histopathology of naturally affected sheep from other sections, although both are clearly scrapie. Correlation between histopathological changes and the clinical syndromes was observed. This may be due to different strains of the virus. Additional studies in this area are being carried out at the National Animal Disease Laboratory, Ames, Iowa.

Specimens received from Mission have enabled Diagnostic Services, National Animal Disease Laboratory, Ames, Iowa, to develop additional laboratory procedures to confirm the diagnosis of scrapie.

No scrapie inoculation work is being conducted at Mission; however, tissues from the natural cases observed there are being collected for use by research workers at other locations.

It is hoped that the findings at the Field Trial Study will serve as an adjunct to the great deal of information being accumulated by the research scientists working on scrapie and thereby furnish additional guidance for changing program procedures as well as contributing knowledge necessary and control and eradicate this disease.

REPORT TO COMMITTEE ON TRANSMISSIBLE DISEASES OF SHEEP AND GOATS

Sarcosporidiosis is a parasitic infestation of man and animals by organisms of the genus Sarcocystis. Over 50 species of Sarcocystis have been named, but the specific names have been used more as a matter of customer and convenience than from any conviction that they are valid. There is a feeling by many that the various species described are merely variants of the same organism Sarcocystis miescheriana. The most common species mentioned in the literature are S. miescheriana in swine, S. blanchardi in cattle, S. tenella in sheep, S. bertrami in horses, S. linde-manni in man, S. muris in mice and S. rileyi in ducks. The distribution of these parasites is world wide.
Miescher first described this parasite in the muscles of the house mouse in 1843. The parasites appear as cylindereal or spindle shaped cysts called "Miescher's tubes" which may vary in size from microscopic to 1.5 - 5 cm in length depending upon the host or the location within the host. These cysts contain numerous banana shaped spores called "Rainey's corpuscles" which are approximately 12 by 5 microns in size and are clearly visible with the conventional light microscope. The cost wall is composed of two layers. Extensions from the inner wall form septa which divide the cyst into compartments.

Sarcosporidia are generally considered to be protozoan rather than fungal and their mode of transmission is by consumption of muscle and/or feces and urine of infected animals. This parasite is not considered very pathogenic in that light or moderate infections produce no clinical signs of illness. In very heavy infections lameness, weakness, emaciation, paralysis and even death have been reported. Spindler, Zimmerman and Jaquette (1946) observed vomiting, diarrhea, inappetence and temporary posterior paralysis in pigs fed infected muscles, urine or feces.

The cysts contain a powerful endotoxin (sarcotoxin) found to be highly toxic to rabbits, mice, and sparrows.

Twenty cases of sarcosporidiosis have been reported in humans. Lindeman first reported the condition in man in 1868. In most of the reported cases in man the parasite was found accidently at autopsy, since the individual evidenced no symptoms related to the condition while alive.

Diagnosis of the condition is accomplished by detection of the cysts by gross or microscopic examination of striated muscle.

Regulations governing Meat Inspection Program, C&MS, United States Department of Agriculture provide for distruction of all tissues for human consumption exhibiting gross lesions of this condition. When carcasses are found (during P. M. examination of food animals) with visible lesions the carcass is retained for observation and evaluation by a Meat Inspection veterinarian. The disposition of the carcass is based on the extent of the lesions. "If the lesions are localized in such manner and are of such character that the parasites and the lesions caused by them can be completely removed, the non-affected portion of the carcass . . . may be passed for food after removal and condemnation of the affected portions. . . . If parasites are found to be distributed in a carcass in such a manner or to be of such character that their removal and the removal of the lesion caused by them is impracticable, no part of the carcass shall be passed for food. . . . If the infestation is moderate the carcass may be passed for cooking."

Reports of incidence of sarcosporidiosis in ewe sheep slaughtered in federal establishments from September 4-17, 1966, indicate that this condition is found with greater frequency in Texas, California and Nebraska. Contacts with C&MS veterinarians in California and the manager of a meat company at Dixon, California indicate that lots of ewes having the highest incidence of this condition originate or were purchased from Montana, Idaho, Utah, Nevada, Oregon, Washington and California. Packing plant management personnel and C&MS veterinarians in San Antonio
and San Angelo, Texas indicate that the incidence of the condition is highest in ewes originating or purchased from Colorado, Wyoming, New Mexico, and parts of Iowa. During discal year 1965, 8.3 percent of the total condemnations and 1.1 percent of the partial condemnations in sheep and lambs were made for sarcosporidiosis.

There have been no trace backs to date to the farms of origin of these animals.

C&MS has established liaison with Animal Health Division, Agricultural Research Service, and the California Department of Agriculture in Sacramento because of reports of increased incidence in this area. Drs. Page and Smith of the Bureau of Animal Health for the State of California indicate that no contacts have been made by interested parties concerning sarcosporidiosis. Dr. Yeager of the Bureau of Meat Inspection for the State of California reports no increase in the incidence of sarcosporidiosis in ewes. Dr. Clavel of the Animal Health Division reported that no contacts have been made with his office concerning sarcosporidiosis.

There is no known treatment of this condition. Since sarcocystis infections are acquired through fecal and urine contamination of food and drink preventive measures should be aimed at reducing contamination. "Our lack of knowledge about this ubiquitous parasite regarding hosts and distribution may well be due to its pathogenic inactivity.... This lack of pathogenicity has excited the curiosity of pathologists but little enthusiasm for organized study of the problem." (Eisenstein and Innes, 1956.)

A. M. Carey, D.V.M.
Staff Officer, Planning Branch
Livestock Slaughter Inspection Division

SURVEY
SEPTEMBER 4-17, 1966

<table>
<thead>
<tr>
<th>Districts</th>
<th>Total Ewe Slaughtered</th>
<th>Carcasses Ret</th>
<th>Carcasses Cond</th>
<th>Carcasses Cooked</th>
<th>Carcasses trimmed &amp; passed</th>
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</thead>
<tbody>
<tr>
<td>Northeastern</td>
<td>325</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>North Central</td>
<td>4,378</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Southeastern</td>
<td>1,444</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Central</td>
<td>9,880</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>24</td>
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<td>Southwestern</td>
<td>4,538</td>
<td>173</td>
<td>68</td>
<td>0</td>
<td>105</td>
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<tr>
<td>Western*</td>
<td>5,970</td>
<td>515</td>
<td>198</td>
<td>11</td>
<td>290</td>
</tr>
<tr>
<td>Total</td>
<td>30,095</td>
<td>715</td>
<td>268</td>
<td>11</td>
<td>420</td>
</tr>
</tbody>
</table>

*211 cysts in esophagus only carcass not retained.
ARTICLE I—NAME
The name of this Association shall be "The International Animal Health Association," a non-profit organization.

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

ARTICLE III—MEMBERSHIP
There shall be five kinds of members: Official, allied organization, individual, elected regional delegates, and nonvoting juniors.

OFFICIAL MEMBERSHIP
The animal health departments of each state, also the United States, and the Canadian, and Mexican governments, Puerto Rico, the Virgin Islands, and Los Angeles County, California, and of such other governmental units as the Executive Committee may by a two thirds vote approve, shall be eligible to official membership in this Association and be represented on the Executive Committee by the animal health executive official.

ALLIED ORGANIZATION MEMBERSHIP
Any nonprofit organization approved by the Executive Committee that is national in scope and activity and directly concerned with the interests and objectives of this Association as outlined in Article II—Purpose, may be elected to allied organization membership and be represented on the Executive Committee by a duly authorized member of the organization.
Any person engaged in animal health work for Federal, provincial, state, county, or municipal governments, and any other person interested in animal health science or milk and meat hygiene, may be elected to individual membership.

Such elected regional delegates as provided for in Article V—Executive Committee shall by virtue of such election automatically become members of this organization for such term or terms as may be decided by the Executive Committee and shall pay such dues as the Executive Committee may decide.

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

The meetings of this Association shall be annual and special.

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

The Board of Directors shall consist of the officers, with the exception of the Executive Committee. It shall handle the financial, administrative, and internal affairs of the Association during such time as the Association and/or the Executive Committee is not in session. It shall handle all other duties and responsibilities as may be assigned to it by the Executive Committee or as may be provided in the Constitution. The Board of Directors shall meet immediately after the adjournment of each annual meeting of this Association and at the same place. The purpose of such meeting is to review plans for the administrative functions of the Secretary for the coming year, to give administrative guidance to the Secretary, and to approve the operations of the office of the Secretary. The Board of Directors may meet at such other times and places as it, by a majority vote, deems necessary. The Secretary shall keep minutes of all meetings of the Board of Directors, and after approval of such minutes by the President, they shall be presented to the Executive Committee at the next annual meeting of this Association.
The Executive Committee shall be composed of the executive officer representing the animal health departments of the various states, the principal animal health officer of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive animal health officer of Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, and of such other governmental units as may be approved for official membership by the Executive Committee, the elective officers of this Association, not more than eight (8) delegates at large representing the livestock industry, including poultry, and allied organization members.

No more than two delegates at large from each of the four districts of the United States shall be elected. Said districts shall be known as the Northeast, consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont; the North Central, consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; the Southern, comprising the States of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico, and the Virgin Islands; the Western district, consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select not more than eight nominees for delegates at large. Said nominees shall be selected from and represent the livestock industry, including poultry. No more than two (2) delegated at large shall be elected from each of the four designated areas or districts. Nominations from the floor of the convention may be made for additional nominees by districts, and they shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this Association.

The elected officers shall have the authority to place before the Executive Committee applications for allied organization membership. Not more than five (5) such applications shall be presented to the Executive Committee for consideration at any annual meeting of the International Animal Health Association.

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

The President-Elect shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary for the Association. The Secretary shall receive such salary and allowance as may be fixed by the Executive Committee.
The Executive Committee shall cause to be audited annually, or oftener if deemed necessary, the receipts and disbursements of the Secretary and of the Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall accept or reject applications for individual and for allied organization membership properly placed before it. Three negative votes shall disqualify for either such membership. That, with the exception of a change in the name of this Association, upon the dissolution of this corporation or the termination of activities thereof, all remaining assets thereof shall be contributed for utilization in the advancement of research of diseases of animals, and no part of the net assets shall inure to any person or group of persons for private gain.

ARTICLE VI—PROGRAM COMMITTEE

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

ARTICLE VII—DUTIES OF OFFICERS

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

The President shall officially represent this Association in such places and at such meetings as he, with the concurrence of a majority of the Board of Directors, deems desirable or necessary in the best interests of this Association. He may at his discretion designate a member of the Executive Committee to substitute for him. A report of such attendance shall be made annually to the membership, and all actual expenses incidental thereto shall be paid by this Association.

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

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

4. Second Vice-President: The Second Vice-President shall assume the duties of the President in the event of the absence, disability, or resignation of the President, President-Elect, and First Vice-President. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability, or resignation of the President-Elect and First Vice-President. He shall be an ex-officio member of the Executive Committee and of the Board of Directors.

5. Secretary: The Secretary shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee, he shall publish said proceedings and distribute them to the members of the Association. The Secretary shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association.

He shall keep an accurate account of all Association moneys received and disbursed. All moneys due this Association received by the Secretary shall be promptly turned over to the Treasurer, accompanied by transmittal information identifying the amount, the source, and such other information as the Treasurer and the Board of Directors may require. He shall draw on the Treasurer, on proper warrants, over his signature and that of the President, such sums as may be necessary to discharge the financial obligations of this Association, provided however that for the payment of incidental expenses of his office, the Secretary may draw on the Treasurer from time to time sums not to exceed twenty-five dollars ($25) at any one time on his own authority. He shall also present to the chairman of the Executive Committee a list giving the name, occupation, and address of each applicant for individual membership for the approval of the Executive Committee. He shall prepare forms for applicants for allied organization membership and shall notify each of the elected officers upon receipt of such completed application. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, ex-officio secretary of the Board of Directors, and an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars ($10,000).

6. Treasurer: The Treasurer shall keep an accurate account of all Association moneys received and disbursed. He shall receive from the Secretary all moneys of the Association paid directly to the Secretary, along with proper identification of such moneys. By and with the approval of the Board of Directors, he shall deposit the funds of this Association in such types of accounts as may be approved by
the Board of Directors, and he shall invest the funds of the Associa-
tion or liquidate Association investments in such manner as may be
approved by the Executive Committee upon recommendation of the
Board of Directors. He shall honor warrants for the proper ex-
penditure of Association funds furnished him by the Secretary over
his signature and that of the President. He shall honor warrants from
the Secretary on the Secretary's own authority for incidental expenses
of the Secretary's office in sums not to exceed twenty-five dollars
($25) for any given expenditure. He shall be given guidance and gen-
eral administrative supervision by the Board of Directors, and he
shall furnish the Executive Committee with a financial statement of
the Association's funds annually. He shall be bonded for not less than
ten thousand dollars ($10,000), and he shall receive such salary as the
Executive Committee may from time to time determine.

ARTICLE VIII—AMENDMENTS

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

BYLAWS

ARTICLE I—ORDER OF BUSINESS

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

ARTICLE II—APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing
to the Secretary. The application shall give the name, occupation, and
address of the applicant and shall be accompanied by a fee of ten
dollars ($10.00), which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary, who shall in turn submit them to the Executive Committee.

Applications for allied organization membership shall be made in writing to the Secretary on an appropriate form prepared by him. In turn, notice of receipt of such application shall be provided each of the elected officers.

An individual or allied organization member may be expelled for cause by the Executive Committee. A majority vote by the members of the Executive Committee present and voting shall be required in order to expel any such member.

ARTICLE III—MEETINGS

The annual meetings shall be held in a location selected at a previous annual meeting by a majority of the members of the Executive Committee. The meeting site in the selected location as well as the duration of said meetings shall be determined by the officers of the Association in consultation with the executive officer representing the animal health department of the state in which the meeting is to be held.

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

ARTICLE IV—QUORUM

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

Thirty members of the Executive Committee shall constitute a quorum, providing at least two-thirds of this number are executive officers representing the animal health departments of their respective states.

ARTICLE V—DUES

The dues for individual membership in this Association shall be ten dollars ($10.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary of the Association.

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

The dues for official and allied organization memberships shall be one hundred dollars ($100) each per annum, payable in advance (on or before January 1st each year) to the Secretary of this Association.

This proposed revision was considered by the Executive Committee and unanimously approved on October 12th, 1966.
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There was a total of 92 diagnostic laboratories from 41 states and Puerto Rico that returned the questionnaire appertaining to budgets of the laboratories. Fifty-eight laboratories furnished budget information, and some of these were incomplete. In many cases, budget information was not available to personnel operating the diagnostic laboratories, or the budgets were a portion of a state-wide or university budget and were not determined.

This report will be concerned with those laboratories supplying budget information. Of the 58 laboratories supplying budget information, 31 were affiliated administratively with universities, 15 were affiliated with either state or Federal departments of agriculture, and 10 were independent or industrial laboratories. Budgets were supplied from 43 general laboratories and 15 that accepted only poultry.

The United States of America was divided, for convenience, into eight geographic regions (Figure 1). The initials stand for Pacific Coast States, including Hawaii, Rocky Mountain States, North Central States,
South Central States, Great Lakes States, Northeastern States, and South- eastern States, including Puerto Rico. Total regional budgets are given in Table I. It was undoubtedly difficult for personnel of diagnostic laborator- ies to break down budgets into categories requested, because of many varied situations. Many laboratories would lump three or four categories together, calling the particular item according to their own breakdown budgets. It is thought that the category "Salaries" represented the most accurate information. It is interesting to note that approximately 65 per- cent of total budgets was for salaries.

Considerable levity was undertaken in an effort to draw analogies. Eight diagnostic laboratories affiliated with colleges of veterinary medi- cine submitted rather detailed and complete budget information. The an- nual budgets of these eight diagnostic laboratories totalled $1,186,147, giving an average of $148,271 per diagnostic laboratory. Diagnostic lab- oratories from other colleges of veterinary medicine were supplied but were fragmentary and were not applicable to this type of average. There were 23 laboratories with annual budgets under $50,000, most of these ranging between $25,000 and $30,000. There were 21 laboratories with annual budgets over $100,000. Ten laboratories had annual budgets over $250,000.

According to budget information received, and excluding the National Animal Disease Laboratory at Ames, Iowa, diagnostic laboratory budgets were highest in the State of Georgia and declined respectively in Ohio, California, Wisconsin, Maryland, Pennsylvania, Indiana, Illinois, Dela- ware, Florida, and Michigan. The states listed above reported annual ex- penditures over $200,000, while the first four states (Georgia, Ohio, Cali- fornia, and Wisconsin) reported expenditures over $500,000. It may be well to reiterate that these totals include state-supported, independent, and industrial budgets.

The highest budgets submitted for poultry diagnostic laboratories were, respectively, Georgia, Delaware, California, New Jersey, Arkansas, Pennsylvania, and New Hampshire. The first four states (Georgia, Dela- ware, California, and New Jersey) had budgets over $100,000.

The budget information reported in this paper should be only a start to gain a complete and more accurate representation of both state-wide and regional diagnostic laboratories.
<table>
<thead>
<tr>
<th>Region</th>
<th>Number of Laboratories</th>
<th>Operating Funds</th>
<th>Maintenance Funds</th>
<th>Inventory</th>
<th>Salaries</th>
<th>Travel</th>
<th>Library</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific Coast States*</td>
<td>6</td>
<td>$45,943</td>
<td>$7,060</td>
<td>$16,442</td>
<td>$513,164</td>
<td>$3,440</td>
<td>$590</td>
<td>$586,689</td>
</tr>
<tr>
<td>Rocky Mountain States</td>
<td>7</td>
<td>33,199</td>
<td>7,205</td>
<td>93,370</td>
<td>321,183</td>
<td>4,770</td>
<td>950</td>
<td>469,677</td>
</tr>
<tr>
<td>North Central States</td>
<td>17</td>
<td>271,200</td>
<td>447,041</td>
<td>30,900</td>
<td>826,427</td>
<td>19,325</td>
<td>2,000</td>
<td>1,596,893</td>
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<tr>
<td>South Central States</td>
<td>3</td>
<td>8,000</td>
<td>8,000</td>
<td>1,000</td>
<td>101,000</td>
<td>0</td>
<td>0</td>
<td>138,000</td>
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<tr>
<td>Great Lakes States</td>
<td>7</td>
<td>79,092</td>
<td>140,256</td>
<td>157,655</td>
<td>1,163,048</td>
<td>9,974</td>
<td>700</td>
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<tr>
<td>Northeastern States</td>
<td>5</td>
<td>300,240</td>
<td>800</td>
<td>31,670</td>
<td>926,002</td>
<td>13,100</td>
<td>1,676</td>
<td>1,266,288</td>
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<tr>
<td>Southeastern States**</td>
<td>13</td>
<td>225,177</td>
<td>74,900</td>
<td>90,407</td>
<td>863,066</td>
<td>19,279</td>
<td>3,377</td>
<td>1,309,526</td>
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<tr>
<td>Grand Totals</td>
<td>58</td>
<td>$962,851</td>
<td>$685,262</td>
<td>$421,444</td>
<td>$4,713,890</td>
<td>$69,888</td>
<td>$9,293</td>
<td>$7,217,799</td>
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</table>

*B including Hawaii.

**Including Puerto Rico.
Often times in questionnaires, relatively small, seemingly unimportant portions may reveal a considerably amount of information. Generally a rough picture may be drawn from this which is usually fairly accurate—our case is no exception.

In examining the extra mural activities of the laboratory personnel who completed and returned the questionnaires, some interesting information was obtained. We will look at the findings in order of their appearance in the questionnaire.

I. Professional Meetings Attended—In State—At institution or own expense.

A. State Veterinary Association Meetings

<table>
<thead>
<tr>
<th>Financial Support</th>
<th>100%</th>
<th>Partial</th>
<th>None</th>
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</thead>
<tbody>
<tr>
<td>Director</td>
<td>72.0%</td>
<td>3.4%</td>
<td>11.2%</td>
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<tr>
<td>Assistant</td>
<td>50.0%</td>
<td>6.1%</td>
<td>10.3%</td>
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</table>

B. Scientific Programs

<table>
<thead>
<tr>
<th>Financial Support</th>
<th>100%</th>
<th>Partial</th>
<th>None</th>
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</thead>
<tbody>
<tr>
<td>Director</td>
<td>56.0%</td>
<td>2.3%</td>
<td>5.7%</td>
</tr>
<tr>
<td>Assistant</td>
<td>50.0%</td>
<td>6.1%</td>
<td>4.5%</td>
</tr>
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</table>

C. Livestock and Poultry Meetings

<table>
<thead>
<tr>
<th>Financial Support</th>
<th>100%</th>
<th>Partial</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Director</td>
<td>61.0%</td>
<td>2.3%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Assistant</td>
<td>47.0%</td>
<td>6.1%</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

II. Professional or Scientific Meetings Attended—Out of State—At institution or own expense.

A. National Conference of Veterinary Laboratory Diagnosticians

<table>
<thead>
<tr>
<th>Financial Support</th>
<th>100%</th>
<th>Partial</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Director</td>
<td>37.0%</td>
<td>1.1%</td>
<td>-0-</td>
</tr>
<tr>
<td>Assistant</td>
<td>42.0%</td>
<td>-0-</td>
<td>-0-</td>
</tr>
</tbody>
</table>

B. American Veterinary Medical Association

<table>
<thead>
<tr>
<th>Financial Support</th>
<th>100%</th>
<th>Partial</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Director</td>
<td>31.0%</td>
<td>2.3%</td>
<td>3.4%</td>
</tr>
<tr>
<td>Assistant</td>
<td>36.0%</td>
<td>3.0%</td>
<td>3.0%</td>
</tr>
</tbody>
</table>
C. Poultry Conferences

Financial Support 100% Partial None
Director 34.0% -0- -0-
Assistant 17.0% 1.5% -0-

D. Special Meetings (ACVP, AAAP, Toxicologists, Chemists, Microbiologists, Research Workers, etc.)

Financial Support 100% Partial None
Director 17.0% -0- -0-
Assistant 15.1% -0- -0-

It is easily seen from this section that attendance at out-of-state meetings is just about half that of in-state. There is an even distribution of attendance among all the laboratories, with the commercial group having the highest percentage of attendance.

An interesting finding in reviewing this section of the survey was that the bigger the pie, the smaller the piece. The laboratories in states with numerous branches received much less travel money than those in small states. Also, those laboratories with University affiliations fared better than brethren in Department of Agriculture laboratories.

III. Active Membership in Professional or scientific Organizations—Dues Paid Yes - No

<table>
<thead>
<tr>
<th>Organization</th>
<th>Member</th>
<th>Non-Member</th>
<th>Dues Paid</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVMA</td>
<td>72.8%</td>
<td>27.2%</td>
<td>6.8% 66.0%</td>
</tr>
<tr>
<td>State VMA</td>
<td>51.1%</td>
<td>48.9%</td>
<td>4.6% 46.5%</td>
</tr>
<tr>
<td>Local or Regional VMA</td>
<td>21.6%</td>
<td>78.4%</td>
<td>-0- 21.6%</td>
</tr>
<tr>
<td>NCVLD</td>
<td>15.6%</td>
<td>84.4%</td>
<td>3.4% 12.2%</td>
</tr>
<tr>
<td>USLSA</td>
<td>54.6%</td>
<td>45.4%</td>
<td>3.4% 41.2%</td>
</tr>
<tr>
<td>American College of Veterinary Pathologists and Association of Pathologists</td>
<td>8.5% 91.5%</td>
<td>1.7% 6.8%</td>
<td></td>
</tr>
<tr>
<td>American Association of Avian Pathologists</td>
<td>46.7%</td>
<td>53.3%</td>
<td>6.7% 40.0%</td>
</tr>
<tr>
<td>Other organizations accounting for less than 2.0%</td>
<td>52.0%</td>
<td>48.0%</td>
<td></td>
</tr>
</tbody>
</table>
Now, what may we gather from these figures?

1. The response to the questionnaire shows that people engaged in diagnostic work are interested and concerned with their profession and are not sitting in dark post mortem rooms satisfied to let the world go past them.

2. They are not personally financially fat, as is indicated by the small percentage of people attending meetings at their own expense.

3. Diagnosticians attend as many meetings as time and finances allow. Figures may be misleading in showing 50 percent attendance by directors and 50 percent attendance by assistants, but add them together and they come out to 100 percent for a given laboratory. Thus at least one or two scientific or professional meetings a year are attended by each member of the laboratory staff.

   These meetings are invaluable, not only for the technical information obtained, but for the morale value as well. It is most gratifying and soul satisfying to talk and commiserate with other diagnosticians and discover that they are having the same problems as you and perhaps even some you don't have—as yet.

4. All state institutions are stingy with out-of-state travel money. This is a problem commonly shared by all of us. However, when one considers that there are many other state agencies which are vying for their cut too, we do not fare too badly.

5. Poultry is the magic word. People engaged in poultry diagnostics or research have, by far, more money and travel allowances than any other group. It used to be asked how one might go about getting out of a "chicken outfit." Now perhaps it should be asked how one might go about getting into a "chicken outfit."

6. Diagnosticians are belongers and society joiners. They support their national professional and scientific organizations—72.0 percent AVMA, 70.2 percent USLSA-NCVLD and 46.7 percent AAAP. They also belong to scads of scientific and honorary societies—some of which, doubtless, few people have even heard of.

7. And finally, diagnosticians are poor state association joiners, with 51.1 percent being members. Even worse, they are lousy regarding local veterinary associations, with only 21.6 percent being members.

   It is a shame that so few veterinary laboratory diagnosticians belong to local associations. By belonging to these small groups and attending their meetings, we can educate our fellow veterinarians as to our functions and activities. Only in this way can we show them that we are not feather plucking gut puddlers working in a broken down, dank necropsy room. But rather, we are the good scientists we are, working in clean, well equipped laboratories, using the most modern instruments, and utilizing the latest technical advances to diagnose a myriad of diseases in a mosaic of animals.
C.V.L.D. QUESTIONNAIRE ON LABORATORY FACILITIES

C.V.L.D. LABORATORY QUESTIONNAIRE

ANALYSIS

4. Duties other than Diagnostic (90 labs reporting)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None other than diagnostic</td>
<td>33</td>
</tr>
<tr>
<td>Teaching</td>
<td>22</td>
</tr>
<tr>
<td>Research</td>
<td>32</td>
</tr>
<tr>
<td>Extension</td>
<td>11</td>
</tr>
</tbody>
</table>

5. Administrative Affiliation (85 labs reporting)

<table>
<thead>
<tr>
<th>Affiliation</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>University or College</td>
<td>40</td>
</tr>
<tr>
<td>Regulatory Agency</td>
<td>33</td>
</tr>
<tr>
<td>Commercial</td>
<td>12</td>
</tr>
</tbody>
</table>

6. Type of Work (90 labs reporting)

<table>
<thead>
<tr>
<th>Type</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>61</td>
</tr>
<tr>
<td>Poultry Only</td>
<td>23</td>
</tr>
<tr>
<td>Serology Only</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
</tbody>
</table>

7. Dates of Construction (86 labs reporting) Range: 1886 to 1966

<table>
<thead>
<tr>
<th>Year Range</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1945 and before</td>
<td>19</td>
</tr>
<tr>
<td>1946 - 1950</td>
<td>10</td>
</tr>
<tr>
<td>1951 - 1955</td>
<td>18</td>
</tr>
<tr>
<td>1956 - 1960</td>
<td>18</td>
</tr>
<tr>
<td>1961 to present</td>
<td>21</td>
</tr>
</tbody>
</table>

8. Future Construction Plans (90 labs reporting)

<table>
<thead>
<tr>
<th>Plan</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Future Construction Planned</td>
<td>40</td>
</tr>
<tr>
<td>No Plans</td>
<td>50</td>
</tr>
</tbody>
</table>

9. Total Square Feet of Space (82 labs reporting) Range: 300 to 62,000 sq. ft.

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 or less</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>1,001 - 5,000</td>
<td>40</td>
<td>49</td>
</tr>
<tr>
<td>5,001 - 10,000</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>10,001 - 15,000</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>15,001 - 20,000</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>20,001 or more</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
Almost half of the total laboratories reported total space between 1,001 and 5,000 square feet.

10. Lab Space (80 labs reporting) Range: 170-20,000 square feet

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 or less</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>1,001 - 5,000</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>5,001 - 10,000</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>10,001 or more</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

11. Necropsy Room(s) (81 labs reporting) Range: 0 to 5,200 sq. ft.

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 or less</td>
<td>32</td>
<td>39</td>
</tr>
<tr>
<td>251 - 500</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>501 - 750</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>751 - 1,000</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1,001 - 1,250</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1,251 - 1,500</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1,501 - 1,750</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1,751 - 2,000</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2,000 or more</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Seven laboratories reported no necropsy space and ten laboratories had less than 100 square feet.

12. Isolation (77 labs reporting) Range: 0 to 30,000 sq. ft.

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48</td>
<td>62</td>
</tr>
<tr>
<td>1 - 500</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>501 - 1,000</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>1,001 - 1,500</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1,501 - 2,000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2,000 or more</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

13. Holding Pens (77 labs reporting) Range: 0 to 10,200 sq. ft.

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47</td>
<td>61</td>
</tr>
<tr>
<td>1 - 250</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>251 - 500</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>501 - 750</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>751 - 1,000</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1,001 or more</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>
14. **Cooler Rooms** (79 labs reporting) Range: 0 to 2,500 sq. ft.

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>1 - 100</td>
<td>27</td>
<td>34</td>
</tr>
<tr>
<td>101 - 200</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>201 - 300</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>301 - 400</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>401 - 500</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>501 or more</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

15. **Lab Animal Rooms** (81 labs reporting) Range: 0 to 20,000 sq. ft.

<table>
<thead>
<tr>
<th>Space (Sq. Ft.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>1 - 250</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>251 - 500</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>501 - 750</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>751 - 1,000</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1,001 or more</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

16. **Carcass Disposal** (86 labs reporting)

<table>
<thead>
<tr>
<th></th>
<th>Labs</th>
<th>Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incinerator</td>
<td>64</td>
<td>74</td>
</tr>
<tr>
<td>Other</td>
<td>36</td>
<td>42</td>
</tr>
</tbody>
</table>

Some laboratories reported more than one method of carcass disposal.

17. **Space for Other Functions** - The answers to this question could not be tabulated.

18. **Replacement Value of Buildings** (68 labs reporting) Range: $6,000 - $1,750,000

<table>
<thead>
<tr>
<th>Value (Dollars)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$100,000 or less</td>
<td>29</td>
<td>43</td>
</tr>
<tr>
<td>$100,001 - 500,000</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>$500,001 - $1,000,000</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>$1,000,001 or more</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

At the low end of the scale 13 laboratories (19 percent) gave a replacement value for buildings at less than $25,000.
19. Replacement Value of Laboratory Equipment (77 labs reporting)  
Range: $800 to $600,000

<table>
<thead>
<tr>
<th>Value (Dollars)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$20,000 or less</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>$20,001 - 40,000</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>$40,001 - 60,000</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>$60,001 - 80,000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$80,001 - 100,000</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>$100,001 or more</td>
<td>16</td>
<td>21</td>
</tr>
</tbody>
</table>

Twenty laboratories (26 percent) set a value of $10,000 or less on the replacement value of their laboratory equipment.

20. Value of Capital Lab. Equipment Added Within the Last Three Years (81 labs reporting) Range: 0 to $200,000

<table>
<thead>
<tr>
<th>Value (Dollars)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10,000 or less</td>
<td>52</td>
<td>64</td>
</tr>
<tr>
<td>$10,001 - 30,000</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>$30,001 - 50,000</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>$50,001 - 70,000</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>$70,001 - 90,000</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>$90,001 or more</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Four laboratories had spent nothing on new equipment, 13 laboratories (16 percent) had purchased $1,000 worth or less, and 21 laboratories (26 percent) had bought new equipment costing between $1,001 and $5,000.

21. Equipment Available

Microscopes (90 labs reporting) Range: 1 - 21 microscopes

<table>
<thead>
<tr>
<th>Microscopes (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 4</td>
<td>44</td>
<td>49</td>
</tr>
<tr>
<td>5 - 8</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>9 - 12</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>13 - 16</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>17 or more</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Types of Microscopes (90 labs reporting)

<table>
<thead>
<tr>
<th>Microscope (Type)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverted</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>51</td>
<td>57</td>
</tr>
<tr>
<td>Dissecting</td>
<td>62</td>
<td>69</td>
</tr>
</tbody>
</table>
**Types of Microscopes (cont'd)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase</td>
<td>40</td>
<td>44</td>
</tr>
<tr>
<td>Polarizing</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Darkfield</td>
<td>58</td>
<td>64</td>
</tr>
</tbody>
</table>

**Centrifuges (88 labs reporting) Range: 0 - 14**

<table>
<thead>
<tr>
<th>Centrifuges (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>3 - 4</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>5 - 6</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>7 - 8</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>9 - 10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>11 or more</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

One laboratory did not own a centrifuge.

**Models of Centrifuges (88 labs reporting)**

<table>
<thead>
<tr>
<th>Centrifuge (Model)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table</td>
<td>80</td>
<td>91</td>
</tr>
<tr>
<td>Flood</td>
<td>73</td>
<td>83</td>
</tr>
<tr>
<td>Babcock</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ultracentrifuge</td>
<td>21</td>
<td>24</td>
</tr>
</tbody>
</table>

**Low Temperature Refrigerators (90 labs reporting) Range: 0 - 9 freezers**

<table>
<thead>
<tr>
<th>Freezer (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>6 or more</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Type of Freezer (90 labs reporting)**

<table>
<thead>
<tr>
<th>Freezer (Temp.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20° C.</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>-40° C.</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>-70° C.</td>
<td>42</td>
<td>47</td>
</tr>
<tr>
<td>Dry Ice</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

Some laboratories reported possession of more than one type of freezer.
### Analytical Balance (90 labs reporting) Range: 0 - 6 balances

<table>
<thead>
<tr>
<th>Balance (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>4 or more</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

### Spectrophotometers (90 labs reporting) Range: 0 - 6

<table>
<thead>
<tr>
<th>Instruments (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5 or more</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

### Types of Spectrophotometers (90 labs reporting)

<table>
<thead>
<tr>
<th>Type</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraviolet</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Visible</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Flame</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Fluorometer</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Infrared</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Atomic absorption</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

### Electrophoresis Apparatus (90 labs reporting)

There were 24 laboratories (27 percent) reporting some type of electrophoresis apparatus. The number of laboratories reporting each type was as follows:

<table>
<thead>
<tr>
<th>Electrophoresis (Type)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Gel</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Thin Layer</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Immune</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
Chromatographs (90 labs reporting)

Nineteen laboratories (21 percent) own some kind of chromatography equipment. The types reported were as follows:

<table>
<thead>
<tr>
<th>Type</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Gas</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Thin Layer</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Scintillation Counter - Seven laboratories (eight percent) have this instrument.

Ovens (90 labs reporting)

<table>
<thead>
<tr>
<th>Oven (Type)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying</td>
<td>80</td>
<td>89</td>
</tr>
<tr>
<td>Vacuum Drying</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Paraffin</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>Muffle Furnace</td>
<td>24</td>
<td>27</td>
</tr>
</tbody>
</table>

pH Meter

Sixty-eight of 90 laboratories (76 percent) own this instrument and 22 (24 percent) did not. Forty-one laboratories owned one pH meter, 18 laboratories had two, and nine laboratories had three or more.

Microtitrator and Microtiter

Eleven laboratories (12 percent) owned a microtitrator and nine laboratories (10 percent) had microtiter equipment.

Automatic Pipetting Machine

Fifty-two of 90 laboratories (58 percent) own automatic pipetting machines

Lyophilization Apparatus (90 labs reporting)

Twenty-eight laboratories (31 percent) own this equipment.

Magnetic Stirrers (90 labs reporting) Range: 0 - 15 stirrers

<table>
<thead>
<tr>
<th>Mag. Mix (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>4 or more</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>
Incubators (90 labs reporting)

Two laboratories had no incubators according to their questionnaires.

<table>
<thead>
<tr>
<th>Incubator (Temp.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42° C.</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>37° C.</td>
<td>82</td>
<td>91</td>
</tr>
<tr>
<td>25° C.</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>CO2 TC</td>
<td>25</td>
<td>28</td>
</tr>
</tbody>
</table>

Tissue Culture Roller Drum (90 labs reporting)

Fifteen laboratories (17 percent) have this equipment.

Water Baths (90 labs reporting)

<table>
<thead>
<tr>
<th>Water Baths (No.)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>5 or more</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

Sonicator (90 labs reporting)

Eight laboratories (nine percent) have at least one sonicator.

Photomicrographic Equipment (90 labs reporting)

Forty-four laboratories (49 percent) have this equipment.

Automatic Tissue Processor (90 labs reporting)

Thirty-eight laboratories (42 percent) have at least one automatic tissue processor.

Microtomes (90 labs reporting)

<table>
<thead>
<tr>
<th>Microtome (Type)</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffin</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td>Frozen</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Cryostat</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Sliding</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>
Saws (90 labs reporting)

<table>
<thead>
<tr>
<th>Type</th>
<th>Labs (No.)</th>
<th>Labs (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Bone (as Stryker)</td>
<td>39</td>
<td>43</td>
</tr>
<tr>
<td>Vertebral Splitting</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

Necropsy Table (90 labs reporting)

Twenty-two laboratories (24 percent) reported having mechanical adjustable tables and 68 laboratories (76 percent) fixed tables.

Incinerator (90 labs reporting)

Fifty-eight laboratories (64 percent) have incinerators.

Refrigerated Carcass Storage Room (90 labs reporting)

Thirty-six laboratories (40 percent) have refrigerated rooms.

Dictation Equipment (90 labs reporting)

Thirty-five laboratories (39 percent) reported having individual office equipment and nine laboratories (10 percent) central equipment.

Copying Machine (90 labs reporting)

Fifty-four laboratories (60 percent) had copying machines for letters and 27 laboratories (30 percent) had machines that would handle books.

Adding Machine (90 labs reporting)

Sixty-six laboratories (73 percent) had at least one adding machine. Twelve laboratories had two or more.

Calculator (90 labs reporting)

Thirty-seven laboratories (41 percent) had calculators. Five laboratories had more than one.
CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS

Questionnaire on Laboratory Facilities

1. NAME AND ADDRESS OF LABORATORY: ____________________________
   (Branch laboratory?) ____________________________

2. PHONE: ____________________________
   ____________________________

3. IN CHARGE: DR.__________________ 1st ASSISTANT: DR.__________________

4. DUTIES OTHER THAN DIAGNOSTIC WORK: ____________________________

5. ADMINISTRATIVE AFFILIATION: ____________________________

6. TYPE OF WORK: GENERAL___ POULTRY ONLY___ SEROLOGY ONLY___
   OTHER ______

7. DATES OF CONSTRUCTION: ____________________________

8. FUTURE CONSTRUCTION PLANS: ____________________________
   ____________________________

9. TOTAL SQUARE FEET OF SPACE ______ 10. LAB. SPACE ________

11. NECROPSY ROOM(S) _____ 12. ISOLATION___ 13. HOLDING PENS ___

14. COOLER ROOMS ___ 15. LAB. ANIMAL ROOMS ___

16. CARCASS DISPOSAL: INCINERATOR_____OTHER (SPECIFY)_____

17. SPACE FOR OTHER FUNCTIONS: ____________________________

18. REPLACEMENT VALUE OF BUILDINGS ____________________________

19. REPLACEMENT VALUE OF LAB. EQUIPMENT ____________________________

20. VALUE OF CAPITAL LAB. EQUIPMENT ADDED WITHIN THE LAST 3
    YEARS: ____________________________

21. EQUIPMENT AVAILABLE:

   Microscopes (number) _____

   - Inverted ______
   - Fluorescence ______
   - Dissecting (wide field-
     low power) ______
   - Phase ______
   - Polarizing ______
   - Darkfield ______

   Low Temperature Refrigerators

   - -20° C. _____
   - -40° C. _____
   - -70° C. _____
   - Dry ice _____

   Analytical balance _____
Equipment Available (continued):

**Centrifuges**
- Table Models
- Floor Models
- Babcock
- Ultracentrifuge
  - (Specify)

**Electrophoresis Apparatus**
- Paper
- Gel
- Thin layer
- Immune

**Scintillation counter**

**Chromatographs**
- Paper
- Gas
- Thin Layer

**Ovens**
- Drying
- Vacuum Drying
- Paraffin
- Muffle furnace

**pH Meter**

**Microtitrator**

**Microtiter**

**Automatic pipetting machine**

**Lyophilization apparatus**

**Magnetic stirrers**

**Incubators**
- 42° C.
- 37° C.
- 25° C.
- CO2 Tissue culture incubator

**Tissue Culture Roller Drum**

**Adding Machine**

**Spectrophotometers**
- Ultraviolet
- Visible
- Flame
- Fluorometer
- Infrared
- Atomic absorption

**Water Baths**
- 37° C.
- 43° C.
- 56° C.

**Sonicator**

**Photomicrographic equipment (list)**

**Automatic tissue processor**
- (as Tissuematon)

**Microtomes**
- Paraffin
- Frozen
- Cryostat
- Sliding

**Saws**
- Band
- Bone (as Stryker)
- Vertebral splitting

**Necropsy Table**
- Mechanical adjustable
- Fixed
- None

**Incinerator**

**Refrigerated Carcass storage room**

**Dictation equipment**
- Individual office
- Central system

**Copying machine**
- Letters
- Books
- Calculator
OSTEODYSTROPHIES OF YOUNG PIGS

John C. Peckham, D.V.M.*

INTRODUCTION

Osteodystrophies are diseases characterized by defective bone growth and maintenance resulting from disturbances of bone metabolism. Most osteodystrophies are associated with nutritional imbalances. They may occur at any age but are most common in the young growing individual. Several osteodystrophies require bone growth in their pathogenesis.

Osteodystrophies of swine have been economically important for many years but have increased in importance recently with increased emphasis on efficient utilization of feed and rapid growth rate. In fact, the bony skeleton has not been able to keep pace with the rapid growth of other organ systems.

The rapid rate of development of swine is illustrated by the changes in body weight. Swine reach 17 times their birth weight at eight weeks of age and 40 times birth weight at 16 weeks.

The bone is an active organ especially in growing animals and quickly reflects changes in the general state of health. An example is the abrupt change in growth rate accompanying infectious diseases of young animals.

Normal Bone Formation

Normal growth of bone depends upon a healthy physical state with proper nutrition, adequate exercise, absence of genetic defects, absence of infectious disease, and normal endocrine balance. Disturbances in any of these areas may result in osteodystrophy. Thus, osteodystrophies may be extrinsic or intrinsic in origin.

Bone formation proceeds along two sequences of development depending upon the type of bone formed, intramembranous or intracartilaginous. The bones of the sides and roof of the cranium and most of the facial bones are intramembranous. The other bones of the body form from cartilage by the processes of endochondral and perichondral ossification.

Growth of the bony skeleton after birth is primarily by the process of endochondral ossification occurring at the epiphyseal plate. Normal endochondral growth of bone depends upon uniform sequential changes from cartilage to bone. This sequence consists of chondroblastic proliferation and growth, formation of cartilage columns, provisional mineralization, chondrocytic degeneration, vascular invasion, osteoblastic proliferation, osteoid apposition and finally mineralization (ossification) to form bone. Once this bone is formed, it undergoes internal reconstruction by resorption and apposition along stress lines to support the body weight in its

*Department of Pathology & Toxicology, Pitman-Moore Division, The Dow Chemical Company, Zionsville, Indiana.

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OSTEODYSTROPHIES OF YOUNG PIGS

final form. All through the life of the individual there is continual internal reconstruction of the bone.

Osteoid Apposition

The apposition of osteoid is directly related to the activity of the osteoblasts, and the activity of osteoblasts is related to general nutrition and growth. Therefore, their activity is decreased by lack of any essential nutrients, especially copper, ascorbic acid and possibly manganese. The activity of the osteoblasts is noted at the epiphyseal line where osteoid is deposited after cartilage destruction, leading to formation of mature bone. Osteoblastic activity is excessive in rapidly growing animals and in the region of healing fractures.

Mineralization of osteoid to form bone requires the proper ionic balance of calcium and phosphorus. This balance is under the control of the parathyroid glands and depends primarily upon adequate dietary assimilation of these two minerals. The cardinal lesion of rickets as well as that of osteomalacia is excessive osteoid.

Ossification and Resorption Balance

After mature bone has been formed it still must be reconstructed and maintained. Maintenance of bone requires a balance of uniform ossification and resorption, i.e., as the bone is removed in one area, it is redeposited in another to maintain maximum strength. If ossification exceeds resorption, the bone becomes dense and hard (osteosclerosis). If resorption is excessive, the bone becomes very thin and fragile (osteoporosis). One effect reported in vitamin A deficiency is increased bone metabolism and excessive resorption of bone.

Causes of Osteodystrophy

Abnormal bone formation can result from a large number of specific and nonspecific causes. In general, osteodystrophies may result from any disturbance of bone formation. They may be general, as in nutritional disease, or local as after trauma or osteomyelitis.

Some causes of osteodystrophy have been listed in Table I. The pathologic changes will be discussed in the section on specific osteodystrophies of swine.

PATHOLOGIC STUDIES IN OSTEODYSTROPHY

Clinical Examination—Evaluation of skeletal disease ideally begins while the animal is alive, on its feet and free to move about in the environment. The general features of body conformation can be then noted in more or less natural positions. This is especially important when joint abnormalities are suspected. After the animal is dead, muscle tone changes, articular apposition is lost and clinical bone abnormalities often disappear.

Radiographic Examination—Radiographs are of great value in evaluating bone diseases not only in the live animal but during post mortem
TABLE I
Causes of Osteodystrophy

<table>
<thead>
<tr>
<th>Dietary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin Imbalance</td>
</tr>
<tr>
<td>Vitamin A, D or C Deficiency</td>
</tr>
<tr>
<td>Vitamin A or D Excess</td>
</tr>
<tr>
<td>Mineral Imbalance</td>
</tr>
<tr>
<td>Calcium Deficiency</td>
</tr>
<tr>
<td>Phosphorus Deficiency</td>
</tr>
<tr>
<td>Phosphorus Excess</td>
</tr>
<tr>
<td>Fluorine Excess</td>
</tr>
<tr>
<td>Zinc Excess</td>
</tr>
<tr>
<td>Manganese Deficiency</td>
</tr>
<tr>
<td>Copper Deficiency</td>
</tr>
<tr>
<td>Protein Deficiency</td>
</tr>
<tr>
<td>Protein or Essential Amino Acid Deficiency</td>
</tr>
<tr>
<td>General Malnutrition</td>
</tr>
<tr>
<td>Caloric Restriction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-Dietary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious Disease</td>
</tr>
<tr>
<td>Viral Infection</td>
</tr>
<tr>
<td>Hog Cholera</td>
</tr>
<tr>
<td>Bacterial Infection (Osteomyelitis)</td>
</tr>
<tr>
<td>Brucellosis</td>
</tr>
<tr>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Erysipelas</td>
</tr>
<tr>
<td>Streptococcus, Staphylococcus and Corynebacterium Abscesses</td>
</tr>
<tr>
<td>Hereditary Defect</td>
</tr>
<tr>
<td>Facial Fibrous Dysplasia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hormonal Imbalance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid Glands</td>
</tr>
<tr>
<td>Primary Hyperparathyroidism</td>
</tr>
<tr>
<td>Secondary Hyperparathyroidism</td>
</tr>
<tr>
<td>Anterior Pituitary Gland</td>
</tr>
<tr>
<td>Thyroid Gland</td>
</tr>
<tr>
<td>Gonads</td>
</tr>
<tr>
<td>Androgen</td>
</tr>
<tr>
<td>Estrogen</td>
</tr>
<tr>
<td>Physical Stresses</td>
</tr>
<tr>
<td>Trauma</td>
</tr>
<tr>
<td>Poor Conformation</td>
</tr>
<tr>
<td>Mechanical Immobilization</td>
</tr>
<tr>
<td>Neural Disturbance</td>
</tr>
<tr>
<td>Paralysis</td>
</tr>
<tr>
<td>Nerve Injury</td>
</tr>
</tbody>
</table>
examinations. Lesions are often much easier to evaluate by a good radiograph and much valuable information can be obtained.

**Clinical Pathology**—The metabolic activity of the bone can usually be evaluated from the changes in serum concentrations of inorganic calcium, inorganic phosphate and alkaline phosphatase.

Calcium and phosphates exist in the plasma in amounts very close to saturation. Calcium is present in three forms. About one-half is ionized, while most of the other half is in a colloidal form bound to protein. A small amount is present in non-ionized complexes with acid radicles such as phosphate, sulphate and citrate. These three forms of plasma calcium are in dynamic equilibrium with each other and in homeostatic equilibrium with the extracellular fluids and bones.

Phosphates are present in all the cells and fluids of the body, and are essential in a variety of metabolic activities. They occur in both the plasma and red blood cells in sufficient concentrations to act in both the primary and secondary buffer systems used for regulation of acid base balance.

The regulation of calcium and phosphate metabolism is a very complex process and is not completely understood. Calcium and phosphate have a reciprocal relationship in plasma and the product of their ionic concentrations must be kept below a certain level to prevent precipitation. If calcium becomes elevated, phosphate falls and vice versa.

The maintenance of blood calcium and phosphate levels is primarily the result of parathyroid function either directly or indirectly. Renal re-absorption, intestinal absorption, as well as the metabolism of bone, are additional factors. The skeleton represents an enormous depot of calcium and phosphorus from which these materials may be readily drawn. Because of the homeostatic mechanism, marked bone depletion can occur without appreciable changes in the serum values. For this reason total bone calcium and phosphorus ash determinations often reveal abnormalities not evident in clinical chemistry findings.

Serum alkaline phosphatase is markedly affected by bone growth and bone metabolic activity. At one time, alkaline phosphatase was believed primarily involved in the mechanism of precipitation of calcium phosphate in bone (mineralization). The present evidence indicates that alkaline phosphatase is concerned with elaboration and secretion of protein of the organic matrix (osteoid). The primary defect which results in failure of mineralization appears to be a diminution of their ionic product and physiochemical changes in the matrix.

In fibrous osteodystrophy, serum alkaline phosphatase has been observed to be inversely related to serum calcium (although the changes are delayed when compared to changes of serum calcium).

Blood serum values for 14 normal weanling pigs weighing 7.5 kg. (16.5 lbs) were reported as follows:

- Calcium .... 11.2 mg/100 ml
- Phosphorus .... 4.6 mg/100 ml
- Alkaline phosphatase .... 13.0 sigma units/ml
Analysis of bones from these animals was bone ash 36.8 percent, ash calcium 34.9 percent and ash phosphorus 17.6 percent.

The mean serum calcium for 50 normal six-month-old pigs was reported to be 9.65 mg/100 ml with a standard deviation of ± 0.99 mg.

The mean serum inorganic phosphorus for 43 normal six-month-old pigs was reported to be 10.94 mg/100 ml with a standard deviation of ± 0.98 mg.

Changes in serum calcium and serum phosphate, in serum alkaline phosphatase activity and in bone ash, bone calcium and bone phosphate in osteodystrophy are summarized in Tables II, III and IV.

Necropsy Procedures

Because bone varies in formation and function, it is important that several bones be examined during the post mortem examination, including representatives of the long bones and membranous bones.

A routine necropsy of the pig should include examination of the ribs (especially fifth through ninth ribs), spinal column, sternum, pelvis, scapulohumoral joints, head of humerus, coxofemoral joints, head of femur, femorotibial joints, condyles of femur, proximal articular surfaces of the tibia, facial bones, nasal turbinates, mandible and bone of the cranium.

The equipment needed varies but an essential is a hacksaw or small meat saw. A Stryker bone saw is very helpful in removing the dorsal spinal processes to expose the entire spinal cord and the ventral surface of the spinal canal. A band saw is useful if the volume of work is heavy. Refrigeration of the specimen will often facilitate sawing bone cross-sections.

Cross-sections of the bone are made to observe the density, the alignment of trabeculae and the bone marrow.

It is essential that bone specimens saved for microscopic studies have the bone marrow exposed and be sufficiently thin for good penetration of fixative.

One method for preparing bone is to cut a large slice about one centimeter thick from the desired bone. (Using a specially constructed holding box, bone can be sliced as thin as 3.4 mm. with a band saw.) The one centimeter slice is fixed in 10 percent neutral buffered formalin in a refrigerator at 5°C. After fixation the bone slice is trimmed with a jeweler's saw to about three millimeters thickness for final fixation.

Gross Examination

During the gross examination, the bones should be examined for deformities and fractures. Also, the articular surfaces should be examined. After these external examinations, the bone is sawed longitudinally to expose the epiphyseal line or costochondral junction. These chondral-osteal junctions are examined for uniformity and the relative cartilage-bone ratio. The thickness of the diaphyses and the size of the marrow cavity are also noted. The ribs should be broken and incised at the costochondral to test their density and amount of mineralization. In rickets, the bones are soft and easily incised with a post mortem knife.
### TABLE II
Summary of Changes in Serum Calcium and Phosphate in Osteodystrophy

<table>
<thead>
<tr>
<th>Osteodystrophy</th>
<th>Serum Calcium</th>
<th>Serum Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11.2 mg/100 ml</td>
<td>4.6 mg/100 ml</td>
</tr>
<tr>
<td>Rickets</td>
<td>Decreased</td>
<td>No Change or Decreased</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Decreased</td>
<td>No Change or Decreased</td>
</tr>
<tr>
<td>Fibrous Osteodystrophy</td>
<td>Decreased</td>
<td>Increased</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>Arrest of Growth (Brachy)</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Incomplete Growth Arrest</td>
<td>Decreased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

### TABLE III
Summary of Changes in Serum Alkaline Phosphatase Activity in Osteodystrophy

<table>
<thead>
<tr>
<th>Osteodystrophy</th>
<th>Serum Alkaline Phosphatase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13 sigma units/ml</td>
</tr>
<tr>
<td>Rickets</td>
<td>Increased</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Increased</td>
</tr>
<tr>
<td>Fibrous Osteodystrophy</td>
<td>Increased</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Decreased</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>Increased or Normal</td>
</tr>
<tr>
<td>Arrest of Growth (Brachy)</td>
<td>Decreased or Normal</td>
</tr>
<tr>
<td>Incomplete Growth Arrest</td>
<td>Decreased or Normal</td>
</tr>
</tbody>
</table>

### TABLE IV
Summary of Changes in Bone Ash, Calcium and Phosphate in Osteodystrophy

<table>
<thead>
<tr>
<th>Osteodystrophy</th>
<th>Bone Ash</th>
<th>Bone Calcium</th>
<th>Bone Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td>Normal - 1 mo. (7.5 kg)</td>
<td>39.3</td>
<td>34.9</td>
<td>17.6</td>
</tr>
<tr>
<td>6 mos. (95 kg)</td>
<td>56.4</td>
<td>40.3</td>
<td>17.6</td>
</tr>
<tr>
<td>Rickets</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Fibrous Osteodystrophy</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>Increased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Arrest of Growth (Brachy)</td>
<td>No Change</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>Incomplete Growth Arrest</td>
<td>Increased</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>(Eury)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Individual bones vary greatly in their rate of maturity and severity of response to disease. This response to disease is closely related to their location and function. Therefore, some bones are better selected for gross and microscopic studies than others.

The ribs are very good bones for gross examination. They are readily exposed and have easily observed costochondral junctions. The ribs are among the last bones to undergo cartilage ossification, therefore, they have active epiphyseal lines throughout life. Also, they are subject to the stress of constant movement during respiration. For accurate evaluation, the same ribs should be examined routinely, the fifth through seventh ribs are most satisfactory. These have maximum movement at the costochondral junction in respiration.

The humerus in the pig supports much of the weight of the body especially the heavy mass of the shoulder, neck and thorax. The reconstruction of the humerus is extensive during growth. This cellular reorganization and stress increase the severity of bone lesions, thereby increasing the value of gross examination of this bone.

The femur and tibia are usually less severely affected by bone disease but are good for additional bone samples. Fractures of the femur frequently occur in osteitis fibrosa (fibrous osteodystrophy).

The facial and gnathic bones, including the turbinates and mandible, are important representatives of the membranous bones in swine. The mandibles because of chewing movements are subject to extensive stress. The nasal turbinates are especially susceptible to bone disease leading to "atrophic rhinitis."

The principal gross pathologic changes in osteodystrophy are summarized in Table V.

Histologic Techniques

Bone requires special techniques in processing in order to obtain good histologic preparations. The first requirement is decalcification.

Decalcification can be accomplished by several methods. The best method used by this writer has been a 30 percent formic acid-ion exchange resin solution. Decalcification of three mm. sections was complete in 18 hours. After 18 hours of fixation in formalin solution, thicker sections of bone were trimmed with a sharp single edge razor blade to a thickness of two to three mm. and returned to the decalcifying solution for an additional 24 hours. This technique resulted in rapid decalcification as well as the preservation of excellent cytologic detail. Other techniques include use of 10 percent formic acid solution buffered at pH 4.5 with sodium citrate and electrolytic methods.

The quality of bone sections can be improved by embedding with celloidin and paraffin using a double infiltration technique. The celloidin-paraffin embedding decreases the tendency for separation of the bone from the cartilage and makes sectioning easier. In many instances, routine paraffin embedding gives adequate sections.

Staining of bone is usually adequate with routine hematoxylin and eosin stain, however, longer staining with hematoxylin is usually
TABLE V
Summary of Principal Gross Pathologic Changes in Osteodystrophy

<table>
<thead>
<tr>
<th>Osteodystrophy</th>
<th>Principal Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rickets</td>
<td>Cartilage - Thickened, irregular epiphyseal line. Enlarged costochondral junction.</td>
</tr>
<tr>
<td></td>
<td>Bone - Soft, deformed bone.</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Cartilage - Mature, no change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Soft, thickened.</td>
</tr>
<tr>
<td>Fibrous Osteodystrophy</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Enlarged, increased soft greyish-white tissue in marrow cavity.</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Thin, fragile.</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Thickened, hard, dense.</td>
</tr>
<tr>
<td>Arrest of Growth</td>
<td>Cartilage - Thin, irregular epiphyseal line.</td>
</tr>
<tr>
<td>Brachyochondroplasia)</td>
<td>Bone - No change.</td>
</tr>
<tr>
<td>Incomplete Growth Arrest</td>
<td>Cartilage - Thickened, irregular epiphyseal line. Enlarged costochondral junction.</td>
</tr>
<tr>
<td>(Euryochondroplasia)</td>
<td>Bone - No change.</td>
</tr>
</tbody>
</table>

TABLE VI
Summary of Principal Histopathologic Changes in Osteodystrophy

<table>
<thead>
<tr>
<th>Osteodystrophy</th>
<th>Principal Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rickets</td>
<td>Cartilage - Irregular cartilage columns.</td>
</tr>
<tr>
<td></td>
<td>Bone - Thick osteoid layers on trabeculae.</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Thick osteoid layers on trabeculae.</td>
</tr>
<tr>
<td>Fibrous Osteodystrophy</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Thick osteoid layers on trabeculae.</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Large lacunae, thin trabeculae, thin compact bone.</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>Cartilage - No change.</td>
</tr>
<tr>
<td></td>
<td>Bone - Thick dense trabeculae, thick compact bone.</td>
</tr>
<tr>
<td>Arrest of Growth</td>
<td>Cartilage - Thin, inactive.</td>
</tr>
<tr>
<td>(Brachyochondroplasia)</td>
<td>Bone - Thin, inactive.</td>
</tr>
<tr>
<td>Incomplete Growth Arrest</td>
<td>Cartilage - Irregular cartilage columns.</td>
</tr>
<tr>
<td>(Euryochondroplasia)</td>
<td>Bone - Thin, inactive.</td>
</tr>
</tbody>
</table>
necessary. The mineral deposits can be well visualized with a modified Bock-Hansen stain using Harris hematoxylin for 30 minutes and differentiating the slides individually in glycerin-glacial acetic acid.

**Histopathologic Examination**

The microscopic changes in osteodystrophy involve basically the cellular constituents of bone and cartilage and their activity. The changes of the matrix are usually secondary to these cellular changes. They involve primarily the osteoblasts, osteocytes and osteoclasts but also the endothelium of the capillary buds at the epiphyseal line and, finally, the chondroblasts and chondrocytes. Their reaction to disease is limited to a relatively few number of responses. Common bone lesions observed include:

1. Excessive amounts of osteoid tissue produced by osteoblasts.
2. Excessive amounts of osteoid produced by metaplasia of cartilage and connective tissue.
3. Inadequate amounts of osteoid tissue.
4. Excessive amounts of cartilage as manifested by excessively broad and wide epiphyseal plates.
5. Twisted, compressed, distorted and fractured cartilagenous, osseous and osteoid trabeculae.
6. Enlargement of the osteocytic lacunae.
7. Hypoplasia of the bone marrow.
8. Hemorrhage in the bone marrow and periosseous structures.
10. Thrombosis of blood vessels.
11. Degeneration and necrosis of cartilage.

The principal histopathologic changes in osteodystrophy are summarized in Table VI.

**Osteodystrophic Diseases of Swine**

Osteodystrophies are defined on the basis of their predominating gross and microscopic changes. These changes are usually not limited to a single causative agent but result from complex interactions of mineral and vitamin imbalances with homeostatic mechanisms. The lesions of more than one osteodystrophy may be present in the same animal.

The most common osteodystrophies of swine include rickets, osteomalacia, osteoporosis, fibrous osteodystrophy (osteitis fibrosa), atrophic rhinitis, "arrest of growth" (brachyochondroplasia), incomplete arrest of growth (eurychondroplasia) and osteosclerosis. Experimental osseodystrophies have been produced by a variety of nutritional imbalances including mangagese deficiency, copper deficiency, zinc excess, fluoride excess and poisoning by radioactive isotopes and by heavy metals such as lead and arsenic.

The most common non-dietary osteodystrophy results from hog cholera virus infection. Bacterial infections (osteomyelitis) often resemble
OSTEODYSTROPHIES OF YOUNG PIGS

grossly an osteodystrophy and must therefore be differentiated by bacteriologic and microscopic examinations.

Rickets

The term rickets is applied to osteodystrophies of rapidly growing animals characterized by soft bones, enlarged irregular cartilage plates and excessive cortical and trabecular osteoid. The basic alterations are both a failure of mineralization of osteoid and a failure of mineralization of cartilagenous matrix.

Rickets as the condition is defined in veterinary medicine has a complex etiology involving nutritional imbalances of vitamin D, calcium and phosphorus.

Rickets in its classical form is a disease of infants caused by a deficiency of vitamin D either of dietary or environmental origin (insufficient exposure to ultraviolet light of sunlight). In the human, a vitamin D deficiency may or may not be accompanied by secondary dietary imbalances of calcium or phosphorus.

It should be remembered that the function of vitamin D is to improve the assimilation of calcium from the diet. Only if the calcium-phosphorus imbalance is within a physiologic range can vitamin D function effectively. If insufficient calcium is available in the diet, vitamin D treatment is ineffective.

The essential changes in rickets are in sequence, failure of provisional calcification of cartilage, failure of degeneration of growing cartilage, irregular overgrowth of cartilage, formation of osteoid on persistent cartilage with irregularity of osteo-chondral junctions, overgrowth of structure of the bones.

The cartilages that contribute most significantly to skeletal growth are most severely affected. They include the epiphyseal plates of proximal ends of the humerus and ulna, the distal ends of the radius, ulna and femur, both ends of the tibia, the articular cartilages, the costal cartilages, the cartilages of the cranial base and the cartilage of the mandibular condyles. The gross features are increased depth of the epiphyseal plate and irregularity of osteochondral junctions.

The diaphyses of the long bones are shorter and broader than normal and contain a narrow marrow cavity. Enlargement of joints of the limbs is one of the typical signs of rickets.

Osteomalacia

Osteomalacia is an osteodystrophic softening of the bones caused by a metabolic disorder that results in a continued negative balance of calcium or phosphorus. Vitamin D may also be involved. The bones become softened, fragile and develop deformities. Osteomalacia is a disease of adults and is basically related to rickets.

Histologically, osteomalacia is characterized by active resorption of bone and the presence of excess osteoid. The trabeculae of the spongiosa are reduced in size and number, and for the most part only the central
zones are calcified. The periphery of the trabeculae are composed of pale acidophilic osteoid. Such osteoid seams line the expanded Haversian canals and localized accumulation at places of excessive mechanical stimulation. Resorptive activity is proportional to the number of osteoclasts present. When osteoporosis is superimposed on osteomalacia, compensatory activity is likely to be minimal, the osteoblasts few in number, and the osteoid which they produce scant.

**Fibrous Osteodystrophy**

Synonyms: Osteodystrophia fibrosa, osteofibrosis, osteitis fibrosa and osteitis fibrosa cystica.

Fibrous osteodystrophy is characterized by excessive fibrous tissue both in the periosteum and marrow cavity. It occurs in primary and secondary hyperparathyroidism and may be superimposed on either rickets or osteomalacia. Its occurrence in these cases may result from complication of rickets or osteomalacia by hyperparathyroidism.

Hyperparathyroidism causes rapid osteoclasis and exuberant growth of loose fibrous tissue in the bones. It may result from diets deficient in calcium or which contain a relatively large excess of phosphates. It can also occur secondarily in severe renal disease.

The principal microscopic changes are excessive fibrous tissue with widespread resorption of compact bone by osteoclasts. Initially the connective tissue is highly cellular and lightly fibrillar. Later, the connective tissue matures becoming more fibrous and less cellular. The bone is weakened and hemorrhage occurs in the connective tissue areas. In attempts to strengthen the bone, new subperiosteal bone is formed. This newly formed bone is embryonic in type, and grossly spongy in appearance. The trabeculae are coarsely fibrillar. The new trabeculae are deposited in a radial fashion on the subperiosteal surface thereby increasing the size of the bones. The spaces between them are filled with connective tissue. The new trabeculae may remain uncalcified and persist for long periods or they may be partially calcified only to be again resorbed and replaced in an irregular fashion. The same process continues on the endosteal surface replacing the medulla and marrow with fibrocellular tissue which contains irregular trabeculae.

The flat membranous bones are most severely affected. These include the bones of the upper face and mandible as well as the ribs, scapulae and pelvic girdle. The articular cartilages are often severely distorted due to resorption of the supporting compact bone and its subsequent collapse.

Fibrous osteodystrophy in swine usually develops following rickets or osteomalacia with pathognomonic enlargement of the skull in only a few animals of a litter. The mandible may be affected as well as the basocranium. The head may enlarge with swelling of the alveolar margins of the jaws and loosening of the teeth. The cranial bones have a normal conformation but are soft.

Recent evidence has been presented that atrophic rhinitis is a form of fibrous osteodystrophy resulting from nutritional secondary
Osteodystrophies of young pigs

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Hyperparathyroidism. Atrophic rhinitis was produced experimentally in growing pigs fed diets deficient in both calcium and phosphorus as well as diets with a relative excess of phosphorus. Hypocalcemia was followed by hyperparathyroidism and generalized fibrous osteodystrophy. The worst causes of atrophic rhinitis occurred if dietary calcium was considerably below normal but not low enough to cause any severe retardation of growth.

The manifestations of fibrous osteodystrophy were atrophic rhinitis, deviation of the snout, infolding of the articular cartilage and subperiosteal resorption of bone with lameness and fractures especially of ribs and femur.

Osteoporosis

Osteoporosis is atrophy of the bone caused by a failure or inadequate formation of organic matrix. However, bone that is formed is well mineralized. Osteoporosis is frequently observed as a result of disuse as in paralysis, mechanical immobilization or loss of neurotropism. It also occurs in protein deficiency, starvation and in cachexia secondary to neoplasia and severe granulomatous diseases.

The bones are lighter, brittle and more fragile than normal. The marrow cavity becomes enlarged. Pathologic fractures are frequent. Ash analysis reveals lowered total calcium and phosphorus levels.

Osteosclerosis (Osteopetrosis)

Osteosclerosis is an infrequent osteodystrophic condition in which the bone is denser and harder than normal. This condition is most common as a localized process and is the result of excessive deposition of mineralized bone matrix. Osteosclerotic lines occur as a result of growth arrest in response to poisoning by metallic phosphorus, lead, arsenic and bismuth. Osteosclerosis is also seen in the heavy metal poisonings caused by radioisotopes.

The principal changes consist of dense, thick, well-mineralized trabeculae and thickened compact bone. The diaphyses may be thickened both on the periosteal and endosteal surfaces resulting in thick and enlarged bones.

Arrest of Growth (Brachychondroplasia)

Arrest of growth is a non-specific osteodystrophy occurring as a secondary result of a variety of diseases, especially severe or chronic disease processes. The cartilage zones are thin and vascular invasion is suppressed. An incomplete thin band of osseous matrix is deposited at the distal ends of the cartilage columns. If the individual recovers from the primary condition, normal growth will return. In these cases, the lines of arrested growth are visible by radiographic examination for long periods, up to many years in the human being. It occurs in starvation, bronchopneumonia, influenza, and other severe infectious diseases as well as heavy metal poisoning. The costochondral junctions of young pigs frequently show lesions of growth arrest. Brachychondroplasia (short
cartilage formation) is a term which well describes the gross appearance of the typical narrow epiphyseal plate.

Microscopically, it is characterized by thin cartilage zones at the epiphysis, suppressed vascular invasion and cartilage destruction, thin trabeculae in the spongiosa and suppression of osteoblastic function with deficient bone deposition. Mineralization is normal and osteoid is present as a very thin layer bordering the trabeculae and the cortex.

**Incomplete Growth Arrest (Eurychondroplasia)**

Incomplete growth arrest is another non-specific osteodystrophy similar in etiology to arrest of growth but differing in gross and microscopic appearances. Incomplete growth arrest can be described as eurychondroplasia (broad cartilage formation). It is defined as a non-specific disturbance in endochondral bone formation characterized by broad proliferating and vesicular cartilage zones, suppressed or irregular vascular invasion and cartilage destruction, thin trabeculae in the spongiosa and suppression of osteoblastic function with deficient bone deposition. Mineralization is normal. Osteoid is present only as thin layers of the trabeculae and in the cortex.

Incomplete growth arrest is the result of excessive cartilage growth relative to bone formation. Gross examination reveals broad epiphyseal cartilage plates often confused with rickets. Based on the microscopic appearance, it can be readily differentiated from rickets.

**Vitamin Imbalance Osteodystrophies**

**Vitamin A Deficiency**—The lesions of hypovitaminosis A depend upon the degree of deficiency and stage of maturity of the affected animal.

In moderate or mild deficiency, the result is incomplete growth arrest (eurychondroplasia) with broad epiphyseal cartilage plates. The endothelial cells of the capillary bud lose their ability to invade cartilage and fail to differentiate into osteoblasts and endothelium. Chondrocytes are only slightly affected and continue to grow and multiply resulting in broad cartilage plates. The absence of osteoblasts results in deficient bone deposition, thin trabeculae and thin cortices.

In severe deficiencies, the entire process of endochondral growth is arrested (brachychondroplasia). Chondrocytes fail to grow and multiply. Vascular invasion and cartilage destruction cease. Endothelial cells and osteoblasts fail to differentiate. Bone deposition ceases and thin trabeculae and cortices result.

**Vitamin B Deficiencies**—Deficiencies in B vitamins result in non-specific arrest of growth with brachychondroplasia.

**Vitamin C Deficiency**—Vitamin C deficiency is a disease of man, primates and guinea pigs. These species either lack an essential enzyme for synthesis or synthesize insufficient amounts of vitamin C to meet body requirements. Swine are apparently able to synthesize this vitamin sufficiently for their needs and confirmed vitamin C deficiency has not been
OSTEODYSTROPHIES OF YOUNG PIGS

reported. If it should be found, these animals could be very valuable for basic research.

In vitamin C deficiency, chondrocytic multiplication and growth continue at a normal rate and vascular invasion and cartilage destruction proceed normally. A lattice of thin osteochondral trabeculae forms in the primary spongiosa as a result of suppressed osteoblast function with deficient deposition of bone. In classical scurvy, these thin trabeculae fracture as a result of muscle tension and stress. This is accompanied by extensive hemorrhage and organization to form the characteristic "Trummerfeld" (field of fragments) and "Gerustmark" (medullary framework) described by early German pathologists.

Vitamin D Deficiency—Vitamin D deficiency results in rickets in man and primates. In swine, the role of vitamin D in rickets is less distinct. Almost all cases of rickets in swine are the result of calcium deficiency or relatively low calcium-high phosphorus rations. In these cases, the addition of vitamin D or exposure to the sun is inadequate to prevent osteodystrophy.

Vitamin D deficiency is reported to occur in the northern climates where there is a minimum exposure to ultraviolet rays. The signs of deficiency are loss of appetite, unthrifty appearance, rough haircoat and lameness. Blood-plasma calcium values decrease. Pigs with rickets can be cured on exposure to the sun for 45 minutes a day for two weeks. Colored breeds of pigs are more susceptible to a deficiency than white breeds. Vitamin D deficiency is rare in pigs in the United States. An additional cause of vitamin D deficiency may be explained by the fact that carotenes including vitamin A in large amounts have been found to be antagonistic to vitamin D.

The lesions of rickets were described earlier in this report. Rickets in swine resembles the form known as juvenile rickets in man. The cartilage changes are less prominent than in the infantile form. A diagnosis of rickets must be based on histopathologic changes as well as gross lesions.

Vitamin A Poisoning (Hypervitaminosis A)—Hypervitaminosis A has been reported in man from overenthusiastic vitamin supplementation. The characteristic manifestations are irritability, pain and swelling over the long bones, external cortical thickening of affected bones, and elevated vitamin A concentrations in the blood.

Experimental hypervitaminosis A in rats resulted in increased metabolic activity and rapid aging of the skeleton. I know of no natural cases of vitamin A poisoning in swine.

Vitamin D Poisoning (Hypervitaminosis A)—Hypervitaminosis D has also occurred with overenthusiastic vitamin supplementation. Experimental hypervitaminosis D has been described in rats and rabbits. Initially bone resorption was accelerated, endochondral bone growth was inhibited and there were large accumulations of an abnormal osteoid matrix. The end result was a deformed osseous framework with porous cortical bone,
excessive periosteal and endosteal new bone and a coarse trabecular structure replacing the marrow.

In addition to bone lesions, hypercalcemia results in metastatic calcification of the heart and large blood vessels (especially the aorta), lungs, kidneys and stomach.

**Mineral Imbalance Osteodystrophies**

*Calcium Deficiency*—The metabolism of calcium is closely associated with phosphorus. For practical purpose, a simple or primary dietary deficiency of calcium is rare, it is instead usually accompanied or conditioned by a relative excess of phosphorus. Swine are frequently fed on plant products which contain relatively little calcium and relatively large amounts of phosphorus. Wide calcium-phosphorus ratios with relative deficiencies of calcium occur in such feeds as cereal grains, the seeds of leguminous plants (including beans and peas), tubers and root crops and by-products of milling processes (including bran).

It should be remembered that the addition of bone meal does not change the calcium-phosphorus ratio of a diet. This simply increases the total amount of both minerals in the diet. Calcium carbonate must be added to the ration to increase the calcium without increasing the phosphorus present.

Calcium deficiency may arise from failure of absorption of dietary calcium as well as dietary deficiency. Calcium is absorbed in the proximal portion of the small intestine. Its absorption is facilitated by an acid medium and inhibited in an alkaline medium since acidity promotes and alkalinity inhibits the ionization of calcium complexes. Insoluble complexes may form with phosphates, citrate, oxalate and phytate.

In rapidly growing pigs, calcium deficiency may be a reflection of unusually high calcium requirements for the growing skeleton. The dietary requirements of calcium in growing pigs was formerly 0.8 percent but recently was reported to be 1.2 percent.

The absorption of calcium is also controlled within certain physiological limits by vitamin D. Deficiency of vitamin D results in decreased absorption of calcium.

Excessive excretion of calcium may occur in pregnancy and lactation. Rarely an idiopathic hypercalcuria may occur.

The pathologic changes in calcium deficiency are quite variable depending upon the age of the animal affected and the severity of the deficiency. Calcium deficiency may result in rickets, osteomalacia or fibrous osteodystrophy. Osteoporosis may also occur secondarily.

The microscopic changes of rickets, osteomalacia and fibrous osteodystrophy were previously described. The principal lesions are thick osteoid layers bordering the trabeculae and the cortex. Osteoblastic function and vascular invasion are normal. If secondary hyperparathyroidism occurs, fibrous osteodystrophy is seen. Osteoporosis results in severe cases when resorption of bone is greater than osteoid deposition.
Phosphorus Deficiency—Phosphorus is an essential element in intermediary metabolism of body cells but lesions are expressed chiefly in the skeleton when a deficiency occurs. As with calcium, phosphorus deficiency may occur not only with a dietary deficiency but also from a failure of adequate absorption. Acidity of the intestinal contents favors ionization and absorption while alkalinity favors insoluble complexes. Insoluble phosphate complexes are formed with a number of elements including calcium, beryllium, iron, lead, aluminum, magnesium, manganese and thallium. The daily phosphorus requirement of growing pigs was formerly 0.6 percent but was readily reported to be 1.0 percent.

Excessive phosphorus loss in renal disease is an important cause of osteodystrophy. This occurs in chronic nephritis and hereditary renal diseases. Renal cortical hypoplasia has been described as occurring in association with renal fibrosis osteodystrophy. Several hereditary renal diseases are recognized in man including phosphate diabetes and the Fanconi syndrome (phosphaturia, glucosuria, amino aciduria). The effects of phosphorus deficiency on the skeleton run parallel to those of calcium deficiency with the exception that fibrous osteodystrophy does not occur. In young animals, rickets occurs and in older animals osteomalacia is seen.

Lesions of phosphorus deficiency in rats are identical to those seen in the human infant with rickets.

Manganese Deficiency—Osteodystrophy has been reported in swine resulting from experimental manganese deficiency.

Clinical manifestations of this osteodystrophy were lameness, enlarged hock joints and crooked legs. This bone deformity became apparent only when the pigs reached a weight of 150 pounds. The length of the forelegs and hindlegs was decreased and there was thickening of the carpal and tarsal areas. Marked bowing of the front legs was also seen. Osteodystrophy resulted in 50 to 60 percent of the animals.

Histologic changes were replacement of the cancellous bone in the diaphysis of the ulna by dense fibrovascular connective tissue. The epihyseal cartilage of the radius was thin and serrated in outline. Increased amounts of pale staining matrix were described as occurring adjacent to the proliferating cartilage zone.

Copper Deficiency—Experimental copper deficiency results in osteodystrophy in swine and dogs. It appeared during the fourth week in all of the experimental pigs. The front legs were bowed laterally at the elbows and turned medially at the distal ends. The hind legs were abnormally crooked and lacked rigidity.

Microscopic changes consist of growth arrest (brachychondroplasia) with thin cortices, thin trabeculae, broad proliferating and vesicular cartilage zones and persistence of a lattice or osteochondral trabeculae with deficient bone deposition. These findings suggest the principal disturbance was in osteoblastic function.

Zinc Excess—Excess zinc in an experimental ration for swine resulted in an osteodystrophy characterized by unthriftiness, lameness and arthritis.
The gross lesions primarily involved the articular surfaces with erosions, ulceration, folding and grooving of the cartilage surface. The joint capsules of the shoulder, elbow, hip and stifles were abnormally distended and contained excessive synovial fluid. Zinc metabolism has been shown to have close relationship to calcium metabolism. It is, therefore, not surprising that these gross lesions resemble the articular changes of fibrous osteodystrophy.

**Fluorine Poisoning**—Fluorine poisoning is reported to cause either osteosclerosis, osteoporosis, osteomalacia or rickets depending upon the severity and age of exposure.

The lesions in growing pigs are similar in many respect to rickets. The ends of the long bones and costochondral junctions are enlarged. The epiphyseal plates are increased in depth, softer than normal and yield to pressure of weight bearing. The change at osteochondral junctions appears to result from continued proliferation of chondrocytes which fail to mature and align themselves. Associated with immaturity, there is a reduced amount of cartilagenous matrix and although this appears to calcify normally, the spicules of calcified cartilage of the primary spongiosa are thin and fragile. Large seams of osteoid are deposited on the trabeculae. This osteoid is poorly calcified and the calcification that does occur is not homogenous.

Periosteal hyperostosis may affect all bones but is most severe on the distal bones of the limbs, the pelvic girdle, the ribs, the mandible and lumbar vertebra. The exostoses develop chiefly at sites of tendinous and fascial muscle insertions. The articular surfaces remain normal.

**Heavy Metal Poisoning (Metallic Phosphorus, Lead, Arsenic, Bismuth)**—Poisoning by metallic phosphorus, lead, arsenic and bismuth produces a disturbance of the ossified cartilage which results in thick trabeculae of cartilage. These thick cartilages are later ossified to form thick bone trabeculae, which form rings or lines of increased density on radiographs. These are termed osteosclerotic bands or lines of arrest of growth. In the case of metallic phosphorus, it has been suggested that the phosphorus combines with calcium salts to form irritating non-absorbable bone. The surrounding osseous tissues then undergo hyperplasia in an attempt to encapsulate the damaged bone which results in osteosclerotic bone.

Osteosclerosis is also a manifestation of poisoning by some of the radioisotopes.

**Hereditary Osteodystrophy**

Facial fibrous dysplasia was reported in closely related swine and therefore a genetic factor was suggested. The lesions were primarily limited to the nasal regions of these pigs. Extensive proliferation of the fibrous connective tissues replaced normal bone. Islands of bony tissues were surrounded by large irregularly outlined, multinucleated osteoclasts. Areas of hemorrhage were prominent and accumulations of hemosiderin were found scattered throughout the connective tissues. These
lesions closely resembled fibrous osteodystrophy resulting from hyperparathyroidism.

Hormonal Imbalance Osteodystrophies

Parathyroid Gland Dysfunction—Hyperparathyroidism may be primary, arising from neoplasia of the parathyroid glands or secondarily arising from functional hyperplasia. In both instances, the bone lesions consist of fibrous osteodystrophy as described earlier.

Pituitary Gland—The growth hormone of the anterior pituitary controls the size of the skeleton. Hyperpituitarism results in gigantism. Hypopituitarism results in dwarfism of the proportional type. Many miniature breeds of animals represent genetic hypopituitarism.

Thyroid Gland—The role of the thyroid gland in basal metabolism is also reflected in bone growth. Hypothyroidism results in retarded growth and bone development. Hyperthyroidism results in acceleration of normal maturation in immature animals. In adult animals, osteoporosis results.

Gonads—Both estrogens and androgens accelerate epiphyseal closures and produce skeletal dimorphism. In hypogonadism there is delayed fusion of epiphyses with disproportional growth of tabular bones and failure of sexual differentiation of the pelvis. Hypergonadism results in premature skeletal closure and maturation of skeleton. Many of the skeletal changes associated with old age are related to decreased estrogens and androgens.

Osteodystrophies Caused by Infectious Disease

Hog Cholera—One of the most common causes of osteodystrophy in swine in the past has been associated with hog cholera infection. Infection of weaned pigs results in a disturbance of calcium and phosphorus metabolism manifested by an interruption of bone growth at the costochondral junction. The lesions are of three types: acute, subacute and chronic. The lesions when present are most constant in the fifth through ninth ribs. In 179 cases, gross lesions occurred in 88 percent of the animals. Moderate to severe lesions were found in 47 percent. Chronic lesions occurred in almost 90 percent of the cases which lingered longer than 30 days from the time of infection. Grossly, the lesions appear as an irregular widening of the white line at the costochondral junction. The bone lesions in chronic cases consist of a line of semi-solid bone which transverses the rib about 5 - 10 mm. proximal from the costochondral junction.

These lesions have been associated with a characteristic marked increase in blood phosphorus and a decrease in blood calcium about the sixth day after infection with hog cholera virus.

Microscopic examination of the costochondral junction reveals a markedly enlarged area of mature cartilage cells between the zone of cartilage cell multiplication and the irregular trabecular bone. The
irregularity of this junction of trabecular bone and the zone of lacunar enlargement is quite evident upon gross examination of the affected rib.

It is apparent from these descriptions that the bone lesions are non-specific and represent arrest of growth with eurychondroplasia.

*Osteomyelitis*—Early stages of osteomyelitis result in gross abnormalities of bone resembling localized osteodystrophy. Microscopic examination usually reveals microabscesses. In later stages, gross abscessation and local resorption of bone is more obvious.

**CONCLUSION**

Bone response to metabolic disturbances results in relatively few basic morphologic changes. However, these changes occur in a large variety of combinations which present a challenge to the diagnostician.

The differential diagnosis of specific forms of osteodystrophy require an evaluation of the case history, physical examination, clinical chemistry determinations, gross examinations and microscopic examinations.

If a nutritional disturbance is suspected, three questions need to be answered:

1. Does study of the diet reveal a deficiency or excess of nutrients?
2. Does examination of animals reveal signs of diagnostic changes of an osteodystrophy?
3. Does correction of the dietary imbalance correct the condition?

Finally, the lesions observed in osteodystrophy vary greatly with the same metabolic or nutritional imbalance. Lesions of experimental studies which permit differentiation in theory often fail to occur in the field and those described as typical textbook cases often fail to exist because of complex interacting causative factors.

**SUMMARY**

The most common osteodystrophies of young pigs include rickets, osteomalacia, osteoporosis, fibrous osteodystrophy (ostitis fibrosa), atrophic rhinitis, "arrest of growth" (brachyochondroplasia), "incomplete arrest of growth" (eurychondroplasia) and osteosclerosis. Osteodystrophies may result from a wide variety of dietary and non-dietary causes. The most important dietary causes in swine are calcium and phosphorus imbalances. The most important non-dietary osteodystrophy results from hog cholera virus infection.

The normal development of bone is reviewed and mechanisms leading to osteodystrophy are discussed. The pathologic studies for evaluation of bone disease are presented with the principal gross and histopathologic changes for each osteodystrophy.
REFERENCES

The following references were reviewed for material for this presentation on osteodystrophies and are suggested for supplemental reading. Illustrations of gross and histologic changes in specific osteodystrophies can be found in these texts.


MALNUTRITION AS INFLUENCED BY INFECTION

K. K. Keahey, D.V.M., Ph.D., and C. K. Whitehair, D.V.M., Ph.D.*

East Lansing, Michigan

There are numerous reports in the literature on the metabolism of nutrients in the normal animal, including the pig. Very little information is available on the metabolism of nutrients as influenced by an enteric infection. In man, gastroenteritis is more severe in malnourished children and leads to body protein depletion. Gastroenteritis is a serious problem in both man and animals. In children gastroenteritis is believed to be responsible for five million deaths per year, and efforts to identify a dominating organism have not been successful (Truswell et al., 1964). In diagnostic work it is highly important to ascertain the role and interrelationship between nutritional deficiencies and infectious agents. It is also important to establish which factor is primary in order to initiate proper treatment and preventive measures. Experimental work was undertaken to study the influence of an enteric infection on protein and electrolyte metabolism using young pigs as the experimental animal. This report is on some of the important findings of this research.

EXPERIMENTAL

Thirty three-week old Yorkshire crossbred pigs were used. At 25 days of age one group was fed a six percent casein, semipurified type of ration and the other group a 32 percent casein ration. Each group was subdivided into an uninfected group and a group infected with the transmissible gastroenteritis virus at 54 days of age. The pigs were maintained in individual metabolism cages that allowed quantitative collection of urine, feces and refused feed. The infected pigs were maintained in separate isolation rooms. The paired-feeding technique was used. The pigs were fed the low and high protein rations for three to four weeks before exposure to the TGE infection. The general composition of the rations was crude casein six, or 32 percent; cerelose 79, or 53 percent; complete mineral mixture five percent; lard 10 percent; and a complete vitamin mixture.

The transmissible gastroenteritis (TGE) virus of known pathogenicity was supplied by Dr. E. O. Haelterman, Purdue University. The infected pigs were given orally two ml. of the suspension of the TGE virus.

Analyses

Blood samples were collected from the anterior vena cava, using an anticoagulant, just prior to inoculation with the virus and at selected intervals following inoculation. Hemoglobin values were determined by the

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cyanmethemoglobin method. Packed cell volume values were determined by the micro method. White blood cell counts were made using Turk's diluting fluid and the hemocytometer. Blood smears were made at the time of bleeding, stained with Wright's stain and a differential white cell count was determined.

Sample of urine, feces, feed and serum were analyzed for nitrogen content by the macro-Kjeldahl method. Sodium and potassium were determined in urine, feces and feed using the Model 21, Coleman Flame Photometer. The serum protein fractions were separated by a Spinco, Model R paper electrophoresis system.

At necropsy, routine tissue sections were collected, preserved in Zenker's fixative and stained with hematoxylin and eosin. Special fixatives and stains were employed when desirable. The histologic procedures followed were according to the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics. Daily records as to weight gains, signs and body temperature were maintained.

RESULTS AND DISCUSSION

The infected pigs fed the six percent casein developed signs of vomiting and diarrhea in 15 to 20 hours after inoculation. Infected pigs fed the 32 percent casein ration developed symptoms usually 30 to 36 hours after inoculation. With the onset of TGE symptoms appetite was reduced. The vomiting usually subsided during the second or third day and the diarrhea continued for eight to 10 days. There was no correlation in the body temperature between the infected pigs and the controls.

The growth rates of the pigs on the low-casein ration were lower than those fed the high-casein ration. The pigs fed the six percent casein ration made gradual gains up to the time of infection and decreased slightly after infection. Hemoglobin, packed cell volumes, and mean differential leukocyte counts were not significantly different between pigs fed the different rations or between the infected and control groups. The leukocyte counts decreased in the infected groups following the inoculation with the TGE virus and were greater in the pigs fed the 32 percent casein ration. Pigs fed six percent casein had lower than normal total serum protein values, and there was no difference between the control pigs and the infected groups.

Nitrogen, Potassium and Sodium Balances

The nitrogen balance data is summarized in Table I. Uninfected pigs fed 32 percent casein were in positive balance throughout the trial. The infected pigs that were fed 32 percent casein also remained in positive balance, but there was a decrease in the amount of nitrogen retained. Control pigs fed the six percent casein ration maintained a slightly positive nitrogen balance. The infected pigs fed six percent casein ration were in negative nitrogen balance at the end of the collection periods on the third and ninth days.

The potassium and sodium balance data are summarized in Table II and Table III, respectively. The normal values for these electrolytes vary
### TABLE I
Nitrogen Balance* of Infected and Control Pigs Fed a Six or 32 Percent Casein Ration

<table>
<thead>
<tr>
<th>Ration</th>
<th>Number of Pigs</th>
<th>Treatment &amp; Number of Pigs</th>
<th>Before Infection</th>
<th>After Infection</th>
<th>Nitrogen Grams Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>32% casein</td>
<td>Uninfected (5)</td>
<td>32% casein</td>
<td>32.2</td>
<td>36.6</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>Infected (10)</td>
<td></td>
<td>34.7</td>
<td>25.3</td>
<td>20.1</td>
</tr>
<tr>
<td>6% casein</td>
<td>Uninfected (5)</td>
<td>6% casein</td>
<td>8.5</td>
<td>6.8</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Infected (10)</td>
<td></td>
<td>6.1</td>
<td>-0.8</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Intake nitrogen Gm. - (urinary nitrogen Gm. + fecal nitrogen Gm.).

### TABLE II
Potassium Balance* of Infected and Control Pigs Fed a Six or 32 Percent Casein Ration

<table>
<thead>
<tr>
<th>Ration</th>
<th>Number of Pigs</th>
<th>Treatment &amp; Number of Pigs</th>
<th>Before Infection</th>
<th>After Infection</th>
<th>Potassium Mg. Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>32% casein</td>
<td>Uninfected (5)</td>
<td>32% casein</td>
<td>3274</td>
<td>3907</td>
<td>4950</td>
</tr>
<tr>
<td></td>
<td>Infected (10)</td>
<td></td>
<td>3884</td>
<td>2663</td>
<td>2508</td>
</tr>
<tr>
<td>6% casein</td>
<td>Uninfected (5)</td>
<td>6% casein</td>
<td>2483</td>
<td>3716</td>
<td>1874</td>
</tr>
<tr>
<td></td>
<td>Infected (10)</td>
<td></td>
<td>2337</td>
<td>-321</td>
<td>2319</td>
</tr>
</tbody>
</table>

*Potassium intake mg. - (urinary potassium mg. + fecal potassium mg.).

### TABLE III
Sodium Balance* of Infected and Control Pigs Fed a Six or 32 Percent Casein Ration

<table>
<thead>
<tr>
<th>Ration</th>
<th>Number of Pigs</th>
<th>Treatment &amp; Number of Pigs</th>
<th>Before Infection</th>
<th>After Infection</th>
<th>Sodium Mg. Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>32% casein</td>
<td>Uninfected (5)</td>
<td>32% casein</td>
<td>1688</td>
<td>2747</td>
<td>2972</td>
</tr>
<tr>
<td></td>
<td>Infected (10)</td>
<td></td>
<td>1905</td>
<td>2031</td>
<td>2219</td>
</tr>
<tr>
<td>6% casein</td>
<td>Uninfected (5)</td>
<td>6% casein</td>
<td>1027</td>
<td>2010</td>
<td>1140</td>
</tr>
<tr>
<td></td>
<td>Infected (10)</td>
<td></td>
<td>1179</td>
<td>-288</td>
<td>1519</td>
</tr>
</tbody>
</table>

*Sodium intake mg. - (urinary sodium mg. + fecal sodium mg.).
considerably, but there were slightly lower balance values for animals fed the low protein ration. Infected animals fed six percent casein were in negative balance for both sodium and potassium 72 hours after inoculation. The increase in fecal water probably accounted for most of the loss in the water soluble electrolytes.

Gross Tissue Changes

The most obvious change between pigs fed 32 percent and six percent casein rations was growth difference (Figure 1). The skin became dry and scaly, and the hair was long in pigs fed six percent casein ration for over four weeks. Approximately one-third of the animals on six percent casein ration had hyperkeratotic lesions of the skin. The most outstanding lesions of the internal organs were those of massive necrosis of the liver. These lesions were most extensive in pigs fed six percent casein and infected with TGE (Figure 2). Control pigs fed six percent casein ration had similar, but less extensive, lesions (Figure 3). Liver tissue was mottled, with depressed, irregular, dark red areas ranging in size from one mm. to one cm. in diameter. These lesions extended in irregular form throughout the parenchymatous tissues of all lobes of the liver. The more normal liver tissue appeared to be grayish brown, while the damaged tissue was dark red and depressed. No gross changes were noted in the livers of control or infected animals fed 32 percent casein ration.

Figure 1. Uninfected pigs fed 32 percent and six percent casein ration.
Figure 2. The liver of the pig infected with TGE and fed six percent casein ration. The dark depressed regions represent necrosis.

Figure 3. The liver of an uninfected pig fed six percent casein ration.
Microscopic Lesions

There were no uniform microscopic lesions in control pigs or pigs infected with TGE and fed 32 percent casein ration. The skin of control and infected pigs on six percent casein had hyperkeratosis (Figure 4). The lesions of hyperkeratosis were observed in both the control and infected pigs fed the low protein ration, and it is believed they were due primarily to the low protein intake. A secondary deficiency of zinc may have also been involved. Approximately half of the pigs on the low protein ration had foci of myocardial necrosis and myocardial edema; control and infected pigs fed six percent casein had atrophic acinar cells of the pancreas, and the cells contained small numbers of secretory granules; many of the epithelial cells lining the tubules of the kidneys contained pyknotic nuclei; and pigs infected with TGE and fed six percent casein had increased amounts of fat in the cytoplasm of cells of the proximal convoluted tubules, descending branch of Henle's loop, and the larger collecting tubules.

Liver Changes: Control and infected pigs fed six percent casein had many lobules in which the cells toward the periphery were large and contained poorly defined spaces in the cytoplasm. The cells did not contain sufficient quantities of fat or glycogen to account for these spaces. Scattered throughout the liver tissue were groups of lobules adjacent to each other in which the parenchymatous cells were undergoing degeneration. This degeneration was characterized progressively by a condensation of the
Figure 5. Liver changes of an uninfected pig fed six percent casein ration. (1) Hepatic cell regeneration, (2) uniform eosinophilic necrotic hepatic cells, and (3) perinuclear basophilic cytoplasm. Hematoxylin and eosin. x 75.

Figure 6. Liver changes of an uninfected pig fed six percent ration. (1) Centrolumbar collection of blood, (2) perinuclear basophilic condensed cytoplasm, and (3) hydropic degeneration. Hematoxylin and eosin. x 75.
cytoplasm around the region of the nucleus, while in other lobules there were hepatic cells with uniformly dense and strongly eosinophilic cytoplasm in which there was either pyknosis, karyorrhexis, or karyolysis of the nucleus (Figure 5). Those lobules of the liver that had a greater quantity of necrosis had lysis of hepatic cells, and the resulting space was filled with engorged blood-filled sinusoids. The filling of the sinusoids with blood gave the appearance of centrolobular hemorrhage (Figure 6). There were increased numbers of fibroblasts and increased amounts of connective tissue in interlobular regions, especially abundant in the vicinity of the portal triads. With the death of the parenchymatous tissue, there was gradual replacement of the lobular region with connective tissue. Therefore, the lobular area became smaller. If the necrotic lobules were located near the capsule of Glisson, there was an indentation of the capsule (Figure 7). The average number of adjacent lobules undergoing degeneration was from three to 10. The predominant type of necrosis of the hepatic cell was coagulation necrosis, which later evidently progressed into liquefaction necrosis. Some of the liver lobules had hepatic cells that contained many mitotic figures, enlarged nucleoli, and cells containing two or more nuclei, which suggested liver regeneration. Necrosis was more extensive in infected pigs fed a six percent casein ration. In some animals there was an extensive increase in interlobular connective tissue in which there were increased numbers of bile ducts (Figure 8). The bile canaliculi were often distended with bile. Sections of liver tissue

Figure 7. Indentation of the capsule of Glisson by hepatic necrosis. An uninfected pig fed six percent casein ration. Hematoxylin and eosin. x 75.
were positive with Best's carmine stain, with the exception of those lobules undergoing necrosis, which were negative. Fat stains (Sudan IV) were negative for liver sections of infected and control pigs fed 32 percent casein and were strongly positive in all infected and control animals fed six percent casein that had extensive interlobular fibrosis. The fat content was greater proportionately with the increase in interlobular connective tissue. These liver lesions are similar to those reported by Obel (1953) in a study of a naturally occurring swine disease in Europe and this liver lesion was termed "hepatosis diaetetica." In recent years this disease has been noted in most swine raising countries in the world. The liver lesions are also similar to a deficiency in the rat described by Schwarz (1960) and called "necrotic liver degeneration" which is believed due to the lack of (factor 3) or selenium. The interrelationship between a deficiency of selenium and vitamin E in the production of this lesion in the pig is not clear. The content of selenium in the experimental rations fed is not known. A dietary source of vitamin E (DL alpha tocopherol) was included in the diet fed at a level of 11.7 mg./kg. of ration. The requirements of vitamin E may be greatly enhanced during an infection.

SUMMARY

Pigs infected with the TGE virus and fed low protein rations developed signs earlier and more severe lesions than infected pigs fed a high protein
ration. The most outstanding gross and microscopic lesions were observed in livers of pigs fed the low protein ration, and were even more severe in those pigs infected with TGE. These lesions were characterized by cirrhosis and fatty metamorphosis and massive liver necrosis. There were some reduction in nitrogen retention for a period at least nine days after infection with the TGE virus. The pigs fed a low protein ration progressed into a negative nitrogen balance. Most pigs infected with the virus had a reduction in potassium and sodium retention for three days after inoculation, then returned to normal levels. In this experiment infections tend to enhance the signs and lesions of nutritional deficiencies.

ACKNOWLEDGEMENTS

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REFERENCES

THE RESPONSE OF THE LIVER TO DEFICIENT OR CONTAMINATED DIETS
Adrianne E. Rogers, M.D.*

Significant functional or morphologic abnormalities of the liver are a common result of dietary insufficiency, imbalance, or contamination. The liver is vulnerable to dietary insults because of its blood supply, its active and complex metabolic functions, and its specialized detoxification functions. It receives its major blood supply through the portal vein and is therefore exposed directly to ingested substances as they are absorbed. It may be exposed to such substances in higher concentration than any other tissue.

The liver in adult rats is composed of parenchymal cells which account for 55 to 60 percent of the total number of cells, reticulo-endothelial and connective tissue cells which comprise 35 to 45 percent of the cells, and bile duct cells which account for three to four percent of the cells. Following injury the liver can regenerate rapidly and fully if the supply of nutrients is adequate and if further injury is prevented. Therefore, in a liver recovering from injury one may see an increased number of mitotic figures in parenchymal cells, Kupffer cells, and bile duct cells. This process has been extensively studied experimentally in rat and mouse livers following a partial, usually two-thirds, hepatectomy. Counts are made of mitoses in the cells of interest, and the incorporation of various precursors into nucleic acids is measured. RNA synthesis rises within four to six hours after surgery, and DNA synthesis follows, beginning at 12 to 18 hours, reaching a maximum at 18 to 30 hours, and then decreasing to normal at about one week. The time of onset, duration, and magnitude of these responses depend on many factors including the age of the animal, the time of day at which the observation is made, the nutritional history of the animal, and the amount of liver removed. The mechanism of the initiation and control of this regeneration is not known.

A useful tool in studies of cell division and DNA synthesis is autoradiography using H3-thymidine. Normal adult rat liver contains approximately 0.07 percent to 0.3 percent H3-labeled parenchymal cell nuclei three hours after the intraperitoneal administration of H3-thymidine, one μc per gram. Zero to 0.1 percent of parenchymal cells are in mitosis. Approximately 24 hours after a two-thirds hepatectomy the H3-labeled parenchymal nuclei may increase to as many as 45 percent of parenchymal cells (Figure 1), and the cells in mitosis to five or six percent. If the animals are starved following surgery, regeneration is inhibited until they are fed; it then proceeds normally. In normal animals decreased food intake can depress mitosis and DNA synthesis, and refeeding is followed by a burst of mitosis. Therefore,

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Figure 1. Liver of a rat 48 hours after a two-third hepatectomy. H\textsuperscript{3}-thymi-
dine, one \( \mu \)c per gram of body weight, was given intraperitoneally three hours be-
fore sacrifice. There are many H\textsuperscript{3}-labeled parenchymal nuclei (black straight ar-
row), bile duct cell nuclei (large clear arrow), and endothelial cell nuclei (small
clear arrow). Emulsion autoradiograph, hematoxylin and eosin, X 200.

The nutritional history of the animal is important in evaluating cell di-
vision in the liver.

The morphologic changes in the parenchymal cell following injury may be those of necrosis or of fatty infiltration. Necrosis is seen follow-
ing many types of injury: interruption of the blood supply,\textsuperscript{14} acute bile duct obstruction,\textsuperscript{15} hepatitis,\textsuperscript{16} and ingestion of a protein-deficient diet,\textsuperscript{17} of carbon tetrachloride, of other chemicals,\textsuperscript{18,19} or of a diet containing a variety of toxic plant materials or mold contam-
inants.\textsuperscript{20-22} Accumulation of fat in the liver cells occurs most commonly in dietary deficiency or imbalance but may be seen also following treatment with certain toxins such as ethionine,\textsuperscript{23} ethyl alcohol,\textsuperscript{24} or carbon tetra-
chloride.\textsuperscript{18,19} In nutritional deficiency the fat which is deposited is
primarily neutral fat apparently derived by normal pathways from depot fat.\textsuperscript{25} It accumulates first in small droplets which then coalesce to form fatty cysts. The amount of functional loss in these cells is not known, but they can return to normal function and appearance if an adequate diet is fed.\textsuperscript{26,27} When an injurious agent acts intermittently or continuously over a period of time, parenchymal regeneration may not keep pace with the destruction of cells and permanent damage ensues. Other cellular elements may behave abnormally. Often in chronic injury there is a proliferation of fibrous tissue, blood vessels, and bile ducts which results finally in cirrhosis.

The development of cirrhosis has been studied experimentally particularly in rats. If one feeds growing rats a diet deficient in compounds (lipotropic agents) which contribute and/or transfer methyl groups in the
body, i.e., choline, methionine, folic acid, and vitamin B₁₂, the rats develop a fatty liver over a period of weeks to months. Generally fat is deposited first in the pericentral parenchymal cells (Figure 2) and later in cells throughout the lobule (Figure 3). As the amount of fat present increases, fibrous tissue is laid down around blood vessels (Figure 4) and along the sinusoids. New blood vessels or shunts are formed which connect central veins to other central and to portal veins and which are contained in the fibrous tissue. These fibro-vascular bands then encircle areas of parenchyma with the production of the familiar picture of the nodular, cirrhotic liver (Figure 5). Thus, if the rat is fed the deficient diet for several months to a year there is an orderly progression, which varies in time and severity with the individual animal, from fatty liver to fibrosis and then to cirrhosis, in which the fibrous bands connect with each other, thereby forming nodules of parenchyma. If the deficient diet is then replaced by an adequate diet, the fat disappears from the liver within a few weeks but the increased fibrous tissue remains.

Studies of the pathogenesis of these lesions have been carried out using H³-thymidine in autoradiographs. When the liver fat content reaches three to four times normal, or approximately 20 percent of the wet weight, increased thymidine uptake is found in parenchymal cell nuclei, in nuclei of endothelial cells of both portal and central veins (Figure 4), and in nuclei of cells in the perivascular areas. These cells are a

Figure 3. Liver of a rat fed a lipotrope-deficient diet for 257 days. Cells throughout the lobule contain fat, although there is a greater amount in the pericentral cells. Hematoxylin and eosin, X 100.
mixture of fibroblasts, bile duct cells, and inflammatory cells. There is little histologic evidence of parenchymal necrosis, but the increasing uptake of thymidine as fat content increases presumably represents regeneration following damage to cells. In the cirrhotic liver the uptake of H-thymidine remains high although the final result is a shrunken liver of only one-half to two-thirds the normal weight. This evidence of regeneration is found diffusely throughout the liver at all times in the development of cirrhosis (Figure 6). It has been thought that certain nodules which contain less fat or have other histologic features were "regenerative nodules," that is, areas composed of cells dividing more rapidly than cells in other areas. Results of studies using H-thymidine show no evidence to support this idea. If counts of H-labeled nuclei are made on fatty and non-fatty nodules, they are found to be the same. Fat-containing cells take up H-thymidine and undergo mitosis as do cells more

Figure 4. Liver of a rat fed a lipotrope-deficient diet for 20 weeks and given H-thymidine before sacrifice. There is increased fibrous tissue around the central vein. There is an H-labeled endothelial cell (arrow). Emulsion autoradiograph, hematoxylin and eosin, X 400.
nearly normal in appearance (Figure 7). Therefore, the idea that the growth of certain "regenerative nodules" accounts for the nodularity and the vascular abnormalities of the cirrhotic liver cannot be accepted on the basis of available evidence.

Increased uptake of thymidine is found in the endothelial and perivascular cells as fatty liver develops and continues throughout the development of cirrhosis. Proliferation of these cells apparently contributes to the formation of the fibrous bands. Both the portal and central veins proliferate and are included in the fibrous tissue. There are severe disturbances of blood flow in the cirrhotic liver with a marked decrease in portal vein flow, an increase in portal pressure, and poor perfusion of the sinusoids. The mechanism of development of this obstruction to flow is not completely understood. It may result in part from sinusoidal obstruction by the fat-filled cells and later from the proliferating perivascular fibrous tissue. The proliferation of blood vessels may be a response to this obstruction. There may also be a direct effect of the deficient diet on the vascular tissue.

In rats and in humans with fatty nutritional cirrhosis there are abnormalities in many areas of metabolism. One which is readily demonstrable histologically is an accumulation of iron in the liver in parenchymal, bile duct, and connective tissue cells. Increased iron

Figure 5. Liver of a rat fed a lipotrope-deficient diet for one year. Broad bands of fibrous tissue containing small blood vessels (arrows) enclose areas of parenchyma to form nodules. Hematoxylin and eosin, X 100.
absorption from the gut has been demonstrated with much of the excess iron being deposited in the liver.33

Another area of dietary hepatic injury is that which results from ingestion of toxic substances derived from plants, molds, or fungi present in the food. These compounds may cause hepatic necrosis and death. If the animal survives, the liver may recover completely or it may become cirrhotic. In addition, some of the naturally occurring toxic products may be carcinogenic. We are studying the effects of aflatoxin, which is an hepatic toxin and carcinogen derived from the mold Aspergillus flavus.20,34,35 Aflatoxin administered orally or parenterally causes hemorrhagic necrosis of the liver and of many other organs, the extent of the damage depending on the dose and the species used. In many cases

Figure 6. Liver of a rat fed a lipotrope-deficient diet for 12 weeks and given H3-thymidine before sacrifice. H3-labeled parenchymal cells (arrows) are distributed throughout the lobule from the portal vein on the left to the central vein on the right with no localization in any area. Emulsion autoradiograph, hematoxylin and eosin, X 200.
this is followed by proliferation of the bile ducts. If repeated small doses are given, the liver cells become abnormal in appearance and malignant hepatomas develop\(^{36}\) (Figure 8).

We have studied the acute effects of a sublethal dose of aflatoxin on mitoses and on H\(^3\)-thymidine uptake in the liver cells. Both indices of cell division are markedly depressed within a few hours after administration of the compound.\(^{37}\) In some cases the uptake of H\(^3\)-thymidine by Kupffer cells also is decreased. In \textit{in vitro} systems it has been found that aflatoxin is bound to DNA,\(^{38}\) that it causes chromosome breakage,\(^{39}\) and that it depresses RNA and protein synthesis.\(^{40,41}\)

There are a variety of changes which may be seen in the liver following the administration of aflatoxin. These include clumping of nuclear chromatin (Figure 8), enlargement of the nucleolus, increased

![Figure 7. Liver of a rat fed a lipotrope-deficient diet for 20 weeks and given H\(^3\)-thymidine before sacrifice. A fat-containing parenchymal cell has two H\(^3\)-labeled nuclei. Two H\(^3\)-labeled bile duct cell nuclei are at left. Emulsion autoradiograph, hematoxylin and eosin, X 800.](image-url)
Figure 8. Liver of a rat given aflatoxin B1, three mg per kg, intragastrically six hours before sacrifice. There is clumping of the chromatin against the nuclear membrane. Feulgen, X 800.

Figure 9. Rat fed aflatoxin, one part per million of the diet, for 41 weeks. There are many tumor nodules in the liver.
eosinophilia of the cytoplasm, and a general enlargement of the cell. Finally malignant hepatomas develop (Figure 9). There is some evidence that the carcinogenicity of aflatoxin is increased in livers with fatty nutritional cirrhosis. Further work is needed to clarify this point.

REFERENCES


MYCOTOXICOSES
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Diseases associated with toxins produced by fungi have not received as much attention as those produced by bacterial toxins. This may be due to the more chronic nature of some mycotoxicoses, to a lack of scientific information regarding them, or to their less common occurrence. Animals have been fed "moldy" grain rejected for human consumption without suffering from obvious disease. Thus, in some quarters "moldy feeds" are considered harmless. Indeed, a great many species of fungi have no known deleterious effects; some, however, are known to produce toxic metabolites. Of course, some fungi produce beneficial metabolites such as the antibiotics. We can expect a relative increase in the importance of mycotoxicoses as bacterial diseases are controlled and as more information becomes available in regard to mycotoxic disease. Evidence attesting to this trend is seen in recent research publications and review articles such as Mycotoxins in Foodstuffs, C. N. Wogan, editor, and the review article by Joseph Forgacs in the 1962 proceedings of this conference.

The extensive recent material, especially by Forgacs and by W. T. Carll, make a detailed review unnecessary at this time. This paper will describe some of the recognized mycotoxic diseases with special emphasis on those for which there has lately been new information of practical significance. The diseases and syndromes to be discussed are the following:

1. Disease due to toxic moldy rice,
2. Stachybotryotoxicosis,
3. Alimentary toxic aleukia,
4. Facial eczema,
5. Moldy-feed poisoning,
6. Penicillium rubrum toxicosis, and
7. Aflatoxicosis.

Toxic moldy rice is an important factor to be considered in stored rice in the Orient. Since 1940 considerable research on such rice has been reported from Japan. The toxic metabolites usually are not destroyed by cooking. Fortunately, the moldy grains are usually brittle and are broken by the polishing process and washed away. Also, the grains may be pigmented or "chalky" white and thus can be removed manually. For these reasons the changes of toxicity affecting man are much smaller than in the case of animals.

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Toxin-producing fungi found on rice to date include members of the groups, *Fusarium*, *Rhizopus*, *Aspergillus*, and *Penicillium*. The most significant research has been reported on *Penicillium islandicum*. Feeding of the fungus or of methanol extract results in acute centrilobular necrosis in rats. Long-term feeding results in liver necrosis, cirrhosis, and sometimes adenomatous nodules. The Japanese workers isolated two toxic materials by chemical fractionation. One fraction causes a centrilobular necrosis in the liver while the other produces a perilobular lesion.

Stachybotryotoxicosis results from ingestion of the toxin produced by the fungus, *Stachybotrys atra*. The disease was first recognized, and is apparently most common, in the USSR. The fungus grows on wet straw and, therefore, produces disease during the winter months. Although most common in horses, other species are also affected. According to descriptions of the disease the toxin produces degeneration and necrosis of the hematopoietic tissue. In high doses this results in thrombocytopenia and severe hemorrhage with death in about one day. Edema and necrosis about the mouth apparently result from direct necrotoxic action. In lower doses severe leukocytopenia allows secondary infections to kill the animal in about six days. Necrotic ulcers in the digestive tract result from necrosis of the lymphatic tissue and loss of normal body defenses. Severe fatty degeneration of liver and kidney is observed in the protracted disease.

A syndrome in humans similar to stachybotryotoxicosis is recognized in Russia and is known as alimentary toxic aleukia (ATA). The disease was especially severe during the war and post-war years of 1942 to 1947 when wheat that had overwintered in the field was consumed. ATA is caused by ingestion of one or more toxins produced by fungi which grow during the alternate freezings and thawings of spring. Some people consider ATA to be the specific disease due to the toxin of *Fusarium sporotrichoides*, while others consider it to be due to a rather large number of different toxin-producing fungi. This is a matter of definition since a number of different fungi can produce toxin or toxins capable of causing the basic disease process of acute fulminating hemorrhage or aplastic bone marrow.

Recently it has been learned that facial eczema, a disease of sheep and cattle in New Zealand and Australia, is of fungal origin. The toxin of *Pithomyces chartarum* produces a liver lesion characterized by cholangitis and obliteration of the bile ducts. When the liver damage is severe enough, the syndrome of photosensitization is produced. The classical features of photosensitization, namely, subcutaneous edema and skin necrosis, give rise to the common name, facial eczema.

"Moldy-feed" toxicosis occurs predominately in swine and chickens because of husbandry conditions in the United States; of common domestic animals these two species are most likely to consume moldy feed. Swine may be affected when they eat moldy corn while foraging in harvested or unharvested corn fields. Chickens are affected when they eat contaminated mixed feed or feed that has been spilled on moist litter. A number of
species of fungi have been isolated from field cases. In acute cases, generalized petechial and ecchymotic hemorrhage is the only lesion. Animals that live longer develop edema and sometimes icterus. Bone marrow aplasia occurs in long-standing cases. Some of the toxic species isolated include *Aspergillus flavus* and *Penicillium rubrum*.

It should be pointed out, however, that many fungi that grow in feed do not produce toxic metabolites. Furthermore, many strains of the toxic fungi either do not produce the toxic metabolite or will produce it only under certain conditions. Therefore, isolation and identification of individual fungi do not alone constitute adequate diagnostic procedure.

The more desirable method of diagnosis is the qualitative and quantitative identification of the toxic metabolite in the suspected feed. This is often not possible since only a few of the toxic metabolites have been identified and a practical testing procedure is available for even fewer. We can expect an increasing amount of research information in this respect in the future.

Toxicosis due to the toxin of *Penicillium rubrum* has been associated with moldy corn toxicosis in the southern United States. The most prominent lesion is extensive hemorrhages throughout the body. Icterus and edema are often present. Histologically, hemorrhage and necrosis in the liver and necrosis in the kidney are evident. Crude extracts of cultured fungi produce the same lesions. In acute doses that kill in one or two days, the only lesion may be petechial hemorrhages in the brain. At present, there is not enough basic information available to develop diagnostic procedures like those which have been developed for aflatoxicosis. Research in progress can be expected to establish the basic information necessary for development of these diagnostic procedures.

Aflatoxicosis, a disease due to the metabolites of *Aspergillus flavus*, has received more attention than the other mycotoxins for at least two reasons. First, an economically significant disease affecting turkey poults, ducklings, pigs, and calves in England in 1960 was shown to be due to aflatoxin. Second, Newberne and others have shown that aflatoxin B1 is a potent carcinogen and produces adenoma and carcinoma of the liver in rats and ducks. Aflatoxin B1 is, indeed, probably the most potent carcinogen known at this time and requires no more than 50 parts per billion in the diet to induce liver carcinoma in rats. In common units this is about one gram per 22 tons of feed. It should be emphasized that this may not be the lower limit of concentration necessary to produce carcinoma. As a result of recently accumulated information a logical approach to diagnostic procedures under field conditions can be made.

*Aspergillus flavus* produces four major metabolites, all of which are toxic compounds. They are fairly simple molecules to which the animal generates no antibodies. The four components were referred to by designations applied to fractions according to speed of migration on a thin-layer chromatogram. When the strip is illuminated with UV light two bluish zones, B1 and B2, and two greenish zones, G1 and G2 can be seen. Chemical identification has shown the formulas to be as follows:
AFLATOXIN B1 AFLATOXIN B2

AFLATOXIN G1 AFLATOXIN G2

B1 is the most prevalent and most toxic fraction and, consequently, has received the most attention. Much of the current research is based on material isolated from cultures grown in liquid media. Recent success in synthesis will hopefully provide samples of B1 in more adequate supply.

Susceptibility varies between species, but the young are generally more sensitive than mature animals. In poultry, ducklings are particularly sensitive, turkey poults and pheasant chicks less so, while chickens are rather resistant. In large animals, pigs, sows, and calves are the more susceptible, while cattle are less so. Sheep are quite resistant. In laboratory animals, the ferret is quite susceptible, the rat is less so, and the mouse is quite resistant.

The acute clinical symptoms are nonspecific; they include inappetence, reduced growth, terminal ataxia, tenesmus, and convulsions. Jaundice is sometimes present, particularly in swine and dogs.

The lesions observed post mortem are hemorrhage, edema, necrosis in many organs, icterus, and liver damage. The primary lesion is in the liver. Depending on the species, age, and amount of toxin ingested, the liver lesions include acute necrosis and hemorrhage, cirrhosis, bile duct proliferation, megalocytosis, enlarged nuclei, lymphocyte and plasma cell infiltration, and liver tumors.

In a case of suspected aflatoxin poisoning the first step should be the duckling bioassay as described by the WHO/FAO/UNICEF Protein Advisory Group for the testing of peanut meal intended for human consumption. The duckling is quite sensitive to the toxic effects of mycotoxin. For instance, the test will detect two µg or about 50 parts per billion of aflatoxin B1.

The assay consists of feeding a balanced diet containing the suspected
feed and a control diet known to be free of mycotoxin. In the case of peanut meal the diet is formulated as follows:

<table>
<thead>
<tr>
<th>Percent</th>
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<tbody>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Corn Oil</td>
</tr>
<tr>
<td>Peanut Meal</td>
</tr>
<tr>
<td>Sucrose</td>
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</tbody>
</table>
| Mineral and Vitamin Mixture | More than 60 percent peanut meal causes some liver lesions even when apparently free of mycotoxin. Thus, in order to do an accurate bioassay it is essential to feed a control diet.

The test animal is a one-day-old duckling (White Pekin or Khaki Campbell) that has not received feed or water. Five ducklings should be used for the test group and five for the control group. The ducklings are fed *ad libitum* for seven days or until death. The parameters measured are:

1. Gain in body weight;
2. Liver weight as percent of body weight; and
3. Histologic liver lesions.

![Normal duckling liver showing portal areas (1) and central veins (2). Note lack of well-defined lobules. Hematoxylin and eosin, X 100.](image)
Histologic examination of control and test groups may utilize frozen or paraffin sections. The paraffin sections should be stained with hematoxylin and eosin and the frozen sections with toluidine blue. Sections should be taken from the same area of liver.

The lesions produced in the duckling depend on the amount of aflatoxin present. Moldy peanuts that cause death in two to three days produce severe hepatocyte degeneration, usually focal hemorrhage in the liver, and only minimal bile duct cell proliferation. Grossly, the liver is swollen and discolored yellow. Conclusions should be based on comparison with normal ducklings since the normal duckling liver contains considerable fat. If the aflatoxin level is low enough for the duckling to live beyond the test period, liver cell necrosis and hyperplasia of bile duct cells are severe. The ductular cells occur as clumps or they may be arranged in poorly formed ductules with irregular lumens, sometimes containing finely granular eosinophilic material. Strands of connective tissue occur subjacent to the basal aspect of the ductules. The nuclei are round to oval, vesicular, and contain prominent clumped chromatin. Isolated hepatic cells are scattered among the proliferating ductules. Mitotic figures are frequent in both hepatocytes and ductular cells.

Figure 2. Normal duckling liver showing central vein. Note moderately vacuolated cytoplasm of hepatocytes. Hematoxylin and eosin, X 450.
Figure 3. Normal portal triad in duckling liver. Vein (V), Artery (A), Bile duct (B). Toluidine blue, X450.

Figure 4. Lymphocytes in portal area of normal duckling liver. Hematoxylin and eosin, X 450.
Figure 5. Granulocytopenesis (developing heterophils) in portal area of normal duckling liver. Hematoxylin and eosin, X 450.

Figure 6. Aflatoxin-induced proliferation of ductular cells extending between portal areas. Hematoxylin and eosin, X 100.
Figure 8. Aflatoxin-induced proliferation of ductular cells in portal area of duckling liver. Hematoxylin and eosin, X 600.
Since it is possible that materials other than aflatoxin may give a positive duckling bioassay, diagnosis should be confirmed by a chemical analysis. Silica gel thin-layer chromatography is currently the most important method for the chemical detection, isolation, and estimation of the aflatoxins. The procedure can be obtained from the National Peanut Council. It is probably best for most veterinary diagnostic laboratories to submit samples to laboratories currently performing the chemical analysis. Several United States government laboratories are currently performing the analysis on peanuts or a peanut product, if the suspected feed is peanuts or a peanut product samples may be submitted to any of these laboratories. Recognizing that suspected animal feed is likely to be grains, such as corn or wheat, the central government laboratory has agreed to work with these materials. Since there is not, at present, a routine procedure for grains and they may require different extraction procedures, the results may not be available as fast as in the case of peanuts. The laboratory has requested that the initial samples be 10

Figure 9. Aflatoxin-induced proliferation of ductular cells forming duct-like structures. Hematoxylin and eosin, X 600.
pounds of the ground grain. Smaller samples will probably be adequate in the future. The charge is about $15 per sample.

Procedures other than the method discussed here exist for aflatoxin assay. The chick embryo is used, but the preparation of a crude extract is required. Briefly, this involves the following steps:

1. Removal of fat with petroleum ether;
2. Extraction of the remainder with methanol;
3. Suspension of methanol residue in propylene glycol; and
4. Injection into yolk sac or air cell of fertile chicken embryo.

Due to the need for efficient tests in the food industry there is considerable research concerned with the development of other biotests. Such things as the effect on the eggs and sperm of the marine borer and the effect on specific strains of *Escherichia coli* may prove useful. It can be expected that new tests will be reported in the future in such publications as the Journal of the Association of Official Agricultural Chemists.

This discussion can best be closed with a quotation from Joseph Forgacs. "Although some of the problems to be encountered will indeed be difficult, the elusive fungi causing these toxicoses are quite interesting. It has been established that fungi in general, because of their highly complex biochemical characteristics, are capable of synthesizing a vast array
of chemical compounds under variable conditions; these compounds are frequently highly complex chemically, structurally unrelated, and have diverse physiologic properties. However, with the continued efforts and team work of specialized scientists, the problems of mycotoxicoses should approach solution. The cause of many of the toxicoses that have occurred in the past and perhaps some of the diseases of unknown etiology that are present today may be determined in the future."

REFERENCES


THE HISTOPATHOLOGICAL DIAGNOSIS OF VITAMIN E DEFICIENCY

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Under field conditions, vitamin E deficiency is commonly complicated by other nutritional deficiencies. The complexity which these multiple nutritional deficiencies confer upon field diagnosis is often perplexing. In the case of deficiencies in vitamin E, the situation is compounded due to the varied functions of vitamin E, some of which may be accomplished by related chemical compounds in the feed. The clinicopathologic manifestations of these abnormal functions are superimposed upon varying husbandry practices and climatic conditions. The end result is usually a syndrome, which does not fit neatly within the boundaries of the more exact experimental studies on vitamin E deficiency.

An accurate clinical history is essential in the diagnosis of vitamin E deficiency. Particularly important are the dietary constituents, length of storage of the feed, and the geographical area from which the feed grain came from. Syndromes of vitamin E deficiency may be caused in the field by one or several of the following conditions:

1. Destruction of vitamin E because of prolonged storage of hay and grain. These syndromes thus tend to occur in late winter and early spring.
2. Destruction of vitamin E because of oxidation by a high fish oil (or other highly unsaturated fatty acids) content in feed. Cod liver oil may have a direct toxic effect on muscle metabolism, which is inhibited by a-tocopherol.
3. Deficiency of compounds that may spare vitamin E (selenium, sulfur-amino acids) in diets already low in vitamin E.
4. Low levels of vitamin E in certain dietary constituents, i.e., corn.
5. Environmental stress on muscle tissue (unaccustomed strenuous movement, shipping, excessive cold, and other factors) while on diets low in vitamin E.

Time is undoubtedly a factor in the occurrence of vitamin E deficiencies. The differing metabolic requirements of muscle at varying ages play a role. The maximum growth of muscle probably coincides with the onset of most nutritional myodystrophies. Vitamin E levels present in the fetus at birth and in colostrum must be highly influential in the occurrence of deficiency syndromes.

The function and mechanism of action of vitamin E in the muscle cell are not entirely known. Its anti-oxidant properties are well established.

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including its interrelationship with the anti-oxidant, selenium. Although vitamin E unquestionably prevents peroxidation of fatty acids, it is uncertain to what extent this is responsible for the cytopathology that occurs in avitaminosis E. Current theories involve vitamin E and selenium as co-factors in intermediary metabolism, possibly directly in energy production in oxidative phosphorylation or in synthesis of coenzyme Q.

Some of the tissue lesions in vitamin E deficiency are due to mitochondrial damage from the disturbance in intermediary metabolism. Muscle damage is an example. Other lesions may be caused by damage to phospholipid membranes that is due to excessive lipid peroxidation in the absence of vitamin E. Some of the vascular lesions that occur may be an example. Because vitamin E deficiency is a metabolic disease, it is manifest by a wide variety of tissue alterations. The biochemical origin of these alterations is not always known. Nonetheless, the histopathologic examination of these tissue changes is probably the most useful and available method of confirmation of vitamin E deficiency.

It would be impossible to list all the lesions of vitamin E deficiency in all species in the time allotted. I would prefer to define those tissue changes that are characteristic of vitamin E deficiency in general. Those who think only of myodystrophy as the sole lesion in vitamin E deficiency in ruminants should also be searching for the more subtle changes that undoubtedly occur, notably in the blood and blood vessels.

**HISTOPATHOLOGY**

*Skeletal Muscle*

Early evidence of degeneration (Figure 1) consists of disappearance of striation, central migration of the nucleus, and acidophilia (hyalinization). Increased numbers of sarcolemmal nuclei can be seen in rows at

![Figure 1](image1.png)  
**Figure 1.** Early skeletal muscle degeneration in a two-week-old chick. Hyaline degeneration is obvious. There are splitting and curling of degenerating fibers. Sarcolemmal nuclei are increased in linear arrangement along fiber.

![Figure 2](image2.png)  
**Figure 2.** A later stage of myodegeneration showing the appearance of macrophages (arrow) which phagocytize necrotic muscle protein.
TABLE I
The Manifestation of Naturally Occurring Vitamin E Deficiency in Various Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Syndrome</th>
<th>Age</th>
<th>Tissue first affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicks</td>
<td>Encephalomalacia</td>
<td>2-3 wks.</td>
<td>Cerebellum</td>
</tr>
<tr>
<td></td>
<td>Exudative diathesis</td>
<td>1-3 wks.</td>
<td>Subcutis (thigh area)</td>
</tr>
<tr>
<td></td>
<td>Myodystrophy</td>
<td>1-4 wks.</td>
<td>Thick muscles</td>
</tr>
<tr>
<td>Calves</td>
<td>Nutritional myopathy</td>
<td>4-6 wks.</td>
<td>Skeletal muscles</td>
</tr>
<tr>
<td></td>
<td>(White muscle disease)</td>
<td>(birth-3 mo.)</td>
<td>(intercostal muscles and large</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>muscles of the upper limbs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cardiac muscle</td>
</tr>
<tr>
<td>Lambs</td>
<td>Nutritional myopathy</td>
<td>2-4 wks.</td>
<td>Skeletal muscles</td>
</tr>
<tr>
<td></td>
<td>(Stiff lamb disease)</td>
<td>(birth-3 mo.)</td>
<td>Cardiac muscle</td>
</tr>
<tr>
<td>Swine</td>
<td>Sudden death (Herztod)*</td>
<td></td>
<td>Cardiac muscle</td>
</tr>
<tr>
<td></td>
<td>Hepatosis diaetetica</td>
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<tr>
<td></td>
<td>(Sweden)</td>
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<tr>
<td></td>
<td>Yellow fat disease</td>
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<tr>
<td></td>
<td>Myodystrophy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mink, cats and</td>
<td>Steatitis</td>
<td></td>
<td></td>
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<tr>
<td>foxes</td>
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*Relationship to vitamin E not yet established.

The fiber periphery. These are the basic histologic changes and are present no matter what other lesions are superimposed upon them. It must be remembered, however, that these are the basic changes in other lesions of muscle and are not pathognomonic for vitamin E deficiency. Numerous fat droplets, detectable with Oil Red O, are present within the muscle fiber. The affected fibers split and curl. Later stages (Figure 2) consist of the development of macrophages which phagocytize muscle protein or fat globules.

Myodystrophy is undoubtedly the most common manifestation of vitamin E deficiency. It occurs in chickens, cattle, sheep, swine, dogs and laboratory animals affected with this disease (Table I). Skeletal muscles are most commonly affected, although in calves and lambs lesions may predominate in cardiac muscle. Consistent with its function in the electron transport system, the earliest lesion of vitamin E deficiency in the muscle cell occurs in the mitochondria. Mitochondria undergo marked enlargement and eventually are disrupted (Figure 3). This condition appears to be the cause of muscle cell death.

**Cardiac Muscle**

Degeneration of cardiac muscle is similar to that in skeletal muscle. It may be accompanied by cardiac failure and pulmonary edema. Fat
stains such as Oil Red O are especially helpful in determining early degeneration. Calcification of necrotic areas may appear as plaques on the endocardium.

**Bone Marrow**

Depression of erythropoiesis is common in vitamin E deficiency in chicks (exudative diathesis)\(^5\) and in swine.\(^8\) It results in a normochromic anemia. Multinucleated erythroid precursors occur in the bone marrow.

**Brain**

Purkinje cell necrosis and encephalomalacia occur in chicks (nutritional encephalomalacia) especially on diets high in fish oils.\(^9\) Engorgement of blood vessels of the pia and outer cerebellar layers is followed by edema, hemorrhage, and necrosis. In chicks which survive, reparative gliosis occurs. A characteristic lesion occurs when Purkinje cell dendrites swell and mineralize (Figure 4). This is best seen in large areas of encephalomalacia. Chronic vitamin E deficiency in rats\(^10\) and other animals is characterized by axonal dystrophy with central nervous system signs.

**Blood Vessels**

Swelling of the endothelial cells of terminal arterioles and capillaries occurs in many lesions in vitamin E deficiency. They are characteristic of exudative diathesis in the chick. Thrombosis and proliferation of endothelial buds (Figure 5) are present in severe cases. It has not been determined whether these lesions are due to or are the cause of other

![Figure 3.](image1.png)  
Electron micrograph of degenerating muscle which contains large amounts of glycogen. The mitochondria are markedly enlarged.

![Figure 4.](image2.png)  
Cerebellar lesions of vitamin E deficiency encephalomalacia in the chick. Severe edema of the Purkinje cell layer, gliosis, areas of malacia and calcification of Purkinje cell dendrites are present.
DIAGNOSIS OF VITAMIN E DEFICIENCY

cellular lesions, or whether they occur because of the same metabolic disturbances.

**Fat**

The development of droplets of oily, amorphous brown-yellow material (ceroid) occurs in fat tissue of swine,\textsuperscript{11} mink\textsuperscript{12} (yellow fat disease), and cats (steatitis). It is insoluble in fat solvents, acid fast, negative for iron stains, and slightly basophilic on hematoxylin staining. Inflammatory cells may surround areas of this substance. Ceroid may also be present in Kupffer cells of the liver. Although ceroid is not present in exudative diathesis of chickens, necrosis of fat occurs, which may be due to vascular damage (Figure 6).

**Liver**

Liver necrosis due to vitamin E deficiency has been reported in swine (hepatosis dietetica).\textsuperscript{13} It also occurs experimentally in rats and chicks. The pathogenesis of liver lesions is uncertain. In rats, they are characterized by markedly enlarged mitochondria.\textsuperscript{14} Similar mitochondrial changes occur in chicken muscle.

Liver lesions of sheep on legume pastures have been recorded in California with the suggestion that these lesions were of nutritional origin.\textsuperscript{15} However, it has been shown that selenium-deficient ewes develop centrolobular hepatic necrosis when given one ml. of carbon tetrachloride. Similar ewes given selenium or vitamin E do not develop such lesions.\textsuperscript{16} Hepatotoxicity may be accentuated in sheep deficient of selenium or vitamin E.

Recently, focal liver necrosis in cattle (sawdust liver) has been reported to be due to vitamin E-selenium deficiency.\textsuperscript{17} The authors stressed the occurrence of cytoplasmic hyaline bodies that is also characteristic of other nutritional disturbances; e.g., choline deficiency and

Figure 5. Proliferation of vascular epithelium in an area of muscle degeneration in the chick.

Figure 6. Lesions in the pericloacal adipose tissue. Necrosis of fat cells and alteration of the blood vessels may be seen.
alcohol toxicity. Such hyaline bodies were prominent in the proximal convoluted tubules of the kidney, in the liver, and in muscle.

Other Lesions

Degeneration of odontoblasts has been described in calves given cod liver oil. In New Zealand, an association has been noted between selenium-responsive diseases in sheep and periodontal diseases.

Differential Diagnosis

The clinical history of animals affected with vitamin E deficiency usually leads one directly to the diagnosis of a nutritional deficiency or toxicological excess. This conclusion is made chiefly because the condition is afebrile, it occurs in young animals, and it is present in most animals of a group. In addition to examination of the diet, some important questions to be answered in the clinical history involve: (1) the use of medication, especially parasiticides; (2) changes in grazing, especially in dry seasons when poisonous plants are more dangerous; and (3) the geographic area where affected animals are located including its soil type, plant life, and other factors.

Some conditions, which might be confused with vitamin E deficiency, are listed in Table II. Nutritional myodystrophy is always characterized by pale, spotty areas in muscle. The lesions as previously described are typical. The greatest hazard is differentiation from rare toxicological causes of muscle necrosis, many of which have yet to be described. Muscle necrosis may be caused by chemical injury of the muscle itself (as in plant poisoning; e.g., Cassia spp. in cattle) or by peripheral nerve injury and secondary muscle degeneration.

In chick, the clinical manifestation of vitamin E deficiency depends upon the manner in which the vitamin was destroyed. In rations that have low nutrient value and have been stored for long periods of time, exudative

| Chicks | Avian encephalomyelitis
|        | Vitamin A deficiency
| Calves and lambs | Transport myopathy (cattle)
|                 | Cod liver oil toxicity (sheep)
|                 | Plant poisoning, Cassia spp (cattle)
|                 | Eosinophilic myositis (Cattle)
|                 | Scrapie (sheep)
|                 | Sulfur contamination of pastures*
|                 | Polyarthritis from umbilical infections
| Swine | Gossypol poisoning
|        | Arsanilic acid poisoning

*These conditions may possibly be related to vitamin E deficiency.
diathesis-myodystrophy develops.\textsuperscript{20} In these cases, vascular lesions are superimposed upon muscle necrosis (Figure 7). Encephalomalacia on the other hand develops chiefly in diets high in fish oils or other highly unsaturated oils. In young chicks, nutritional encephalomalacia may be confused with avian encephalitis (epidemic tremor).

Avian encephalitis virus causes degeneration of nerve cell bodies. This is most easily seen in the Purkinje cell layer in the cerebellum. Chromatolysis and necrosis of these cells is followed by glial proliferation which gives the cell structure of the cerebellum a "wind-swept" appearance. Lymphocytic, perivascular cuffing is prominent in avian encephalomyelitis and does not occur in nutritional encephalomalacia. A fluorescent antibody method for the detection of avian encephalomyelitis has been used.\textsuperscript{21}

Incoordination with central nervous system atrophy has been described in vitamin A deficiency in chicks.\textsuperscript{22} Cell necrosis was not seen. It is doubtful if vitamin A deficiency occurs when modern methods of poultry feeding are used.

In cattle and sheep, vitamin E deficiency is manifest as degeneration of skeletal and cardiac muscle. Such cases of nutritional myopathy have been described in North America, Britain, and Northern Europe as white muscle disease, enzootic muscular dystrophy, cod liver oil toxicity, and stiff lamb disease. Many cases of white muscle disease are selenium responsive. They do not respond to vitamin E nor do they have lowered plasma levels of vitamin E. Once muscle degeneration has been diagnosed in cattle and sheep, an adequate history may pinpoint the need for specific biochemical determinations on plasma or muscle. Muth et al.\textsuperscript{23,24} have presented the relationship between selenium-deficient soils and occurrence of white muscle disease.

Sulfur when used in fertilizer on pasture has been associated with severe outbreaks of muscle degeneration. The muscle lesions of such conditions as hypomagnesemia, hypophysosteatemia, and copper deficiency may, in the future, prove to be important in the differential diagnosis of nutritional myopathy of calves and lambs. Myodegeneration nearly identical to nutritional myopathy has been shown to be due to plant poisoning in Texas.\textsuperscript{25} Similar muscle lesions have been known to exist in other areas of the southwestern U.S.A.\textsuperscript{26} Their origin is open to question.

In swine, in addition to myodystrophy,\textsuperscript{6} many conditions related to vitamin E deficiency have been reported.\textsuperscript{27} Most cases have been
isolated geographically, and experimental studies have been unsatisfactory in explaining the relationship of these clinical entities. These include: (1) massive edema in pigs fed on a diet high in cod liver oil, (2) liver necrosis associated with fish diets in Sweden,13 and (3) a syndrome of sudden death, cardiac muscle degeneration and subtle lesions in the skeletal muscles (Herztod). In relating these syndromes to nutritional deficiency, one must eliminate many conditions which are equally as rare in occurrence. Again deficiencies of magnesium, phosphorus, copper, and others may be important.

The finding of pale areas in muscle tissue of young swine on adequate diets is not uncommon in diagnostic laboratories. These cases are isolated, sporadic and of insufficient economic significance to demand the investigation of possible causes.

BIOCHEMICAL ANALYSIS OF PLASMA VITAMIN E

Ultimate proof of the existence of field cases of vitamin E deficiency rests, in part, on the determination of plasma levels of vitamin E or α-tocopherol. Several methods are available including chemical determination and bioassay. The review of Kofler et al.28 is recommended. Most methods involve separation procedures to eliminate interfering substances. Unfortunately, these methods are not within the realm of most veterinary diagnostic laboratories.

CONCLUSIONS

In summary, muscle degeneration and necrosis characteristic of nutritional myopathy may be determined by histopathological diagnosis. Careful attention should be given to examination of the heart, blood vessels, blood cells, brain, and liver. An accurate history is essential. Complete descriptions of muscle lesions have been given by Hadlow29 while the reviews by Blaxter30,31 and Swahn and Thafvelin27 are helpful. Final proof may lie in examination of the serum—not only for levels of vitamin E but for magnesium, phosphorous, and copper. One suspects, however, that clinical response to α-tocopherol injections provides the ultimate satisfaction with any diagnosis of vitamin E deficiency.

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DIAGNOSIS OF VITAMIN E DEFICIENCY

The increased use of chemicals in agriculture and medicine has created an interest in the toxicologic aspects of these agents. Veterinary drugs are used for a number of purposes such as diagnosis, prevention and the treatment of disease; to promote growth; to influence the structure or functions of the animal body in order to improve quality of meat; to improve feed efficiency; and to tranquilize animals during their shipment. In agriculture, pesticides of many different types have been employed to increase the yield of the land. Animals may incidently come in contact with these chemicals. The question of safety of all of these compounds is an important one to all parties concerned. Toxicologists in industry and in the various governmental agencies which deal with drugs and pesticides are cognizant of the potential problems which might arise and they are constantly striving to prevent them.

Once the potential usefulness of an agent has been elicited in the laboratory, investigations to demonstrate safety for its proposed beneficial use are initiated in laboratory animals. The nature and extent of these are determined by the chemical and biological properties of the agent as well as its proposed clinical application. If the agent is to be used only for one or two doses, the safety evaluation may be very limited. If it is to be administered for a prolonged period of time, more extensive testing is necessary. The safety testing necessary for an agent which may accumulate in the tissues as the original drug or its end products can require elaborate testing even though it is only to be given for short periods clinically. The number and types of investigations necessary to assess the safety of a new drug require a scientific judgement of the known properties of the agent and any additional information acquired during the course of the toxicity evaluation.

Toxicology studies of chemicals are conducted with two major goals in mind. First, since any useful drug can be expected to produce toxic effect under certain conditions, it is necessary to define the toxicologic attributes of that drug. Second, once the toxicity has been demonstrated, it is necessary to define the difference between the doses necessary for the desired therapeutic effect and the approximate highest non-toxic dose. Because of the variety of animals which may be treated by a veterinarian, this information should be developed for each species.

Toxicologic investigations generally fall into three categories: acute, subacute and chronic toxicity studies. In acute toxicity studies, a drug
is administered as single doses, some of which are large enough to be lethal. This type of study provides a rapid estimation of the inherent biologic activity of the agent and sometimes may uncover useful types of pharmacodynamic attributes. Species differences may be demonstrated and the relative efficiency of absorption by the several routes of administration may be determined. Early deaths may indicate that the lethal effects are due to pharmacodynamic activity or, if deaths are delayed, that the toxicity may be due to biochemical or anatomic changes. Acute toxicity studies generally are not adequate for the prediction of the safety of new drugs unless supported by longer term studies which include other parameters of observation than death or acute signs of drug effects.

Subacute and chronic toxicity studies, which are usually conducted in the rat and dog, differ mainly in the duration of treatment, the number of animals used at each dose level and the number of dose levels employed. In subacute toxicity studies as many as six, or even more, dose levels may be employed and the size of the doses should vary from near-therapeutic levels to doses that are toxic after the administration of a few daily doses. This type of study permits the proper selection of dosage levels for use in the chronic study in which three levels and a control are usually employed. The three levels used in the chronic study ideally should be: 1) a non-toxic or "no effect" level which is preferably several times the therapeutic dose; 2) a middle dose level at which toxicity may or may not be produced; and 3) a toxic or "effect" level at which toxic effects may become apparent only after drug administration has continued for perhaps half of the full duration of the chronic study. The duration of subacute and chronic toxicity studies may vary from a few days to two years. In unusual circumstances they may be continued for more than two years.

Since a drug is given continuously for periods of days to many months, it is possible to conduct a large number of correlated observations which are not practical in the short term pharmacodynamic studies. The general clinical condition of the animals may be determined by observations of behavior, cardiac and respiratory functions, reflexes and funduscopic examinations. Food consumption and body weight data are important since alterations in these parameters may reflect early toxicity. Hematologic studies and biochemical determinations of the various constituents of plasma and urine may indicate useful drug activity as well as changes due to toxicity. Organ function tests may be employed. A basic battery of tests may be employed in both the subacute and chronic toxicity studies, but, during the toxicity studies, results obtained may point toward adding or deleting tests performed.

Post-mortem examinations are conducted as an integral part of the subacute and chronic toxicity experiments. Gross examinations of the tissues, organ weights and histologic studies occasionally reveal alterations that are not reflected by the other parameters. Since some chemicals may produce only subtle anatomic changes, gross examination of the tissues may not be adequate. A variety of tissues should be examined histologically.
Studies of the metabolism of a chemical are important since the accumulation of that chemical, or its metabolites, in the tissues can complicate the evaluation of safety for use of a chemical in animals which may be a source of food for man. The rate and extent of the absorption and elimination are of great importance. A chemical which is not degraded and is rapidly eliminated by a food animal does not create the problem presented by a chemical which is degraded or eliminated slowly. It is also important to know if the drug alters the metabolism of the body. Thus a drug which might have an effect on the endocrine system could alter the production of eggs or milk, or interfere with reproduction.

Reproduction studies should be conducted since alterations of the reproductive cycle of domestic animals can be of great economic importance. The veterinarian has a great advantage over the clinician in the field of human medicine since it is possible to conduct reproduction studies, as well as routine toxicity studies, in the species for which the drug is intended. Multigeneration studies permit an evaluation of drug effect on several reproductive cycles and may detect genetic alteration.

Another aspect of the safety evaluation of chemicals with which domestic animals may come in contact is not related directly to the safety for the treated animal but rather to the safety for the human who may consume food with chemical residue derived from treated or "accidentally contaminated" animals. The usual safety evaluation for this type of chemical consists of subacute and two year chronic toxicity studies in rats and dogs, with suitable tissue residue studies in the food producing animals. The fact that residues do or do not exist after administration of a specific agent may first be investigated using the chemical synthesized with a radioactive element. If it is determined that a residue is present, methods must be developed for analysis of non-radio-labeled chemical. These methods must be sensitive enough to detect very low levels of residue. Depending on the toxicity of the particular chemical under discussion, tolerances of the order of magnitude of 0.1 ppm for tissues or 0.01 ppm for milk and eggs may be established. Thus, methods of analysis must be developed which are sensitive down to these levels in the particular sample types to be analyzed. The concept of "zero tolerance" which had been advocated previously has had to give way to advances in analytical techniques. What is zero with one analytical technique may indeed be a definite amount using a newer, more sensitive method. The various chromatographic methods, among others, such as gas, thin layer, etc., have been responsible for advances in this area.

In addition to the information on safety and residues, efficacy data for new drugs must also be elicited and submitted to the governmental regulatory agencies. It is not enough that new chemicals be safe to use in and around animals, these agents must also be effective in doing the job for which they are being promoted. This has lead to the widespread utilization of veterinary clinicians for the evaluation of new
compounds in the field. The federal regulations state that a new drug must be clinically evaluated by "experts qualified by scientific training and experience". The results obtained must be documented and submitted to the sponsor of the study for inclusion in their application to the Food and Drug Administration. At this point, information on toxicity which might develop during clinical trials is also fed back to the sponsor and the Food and Drug Administration. A continual feedback of such toxicity information during the entire market life of a drug is of great importance and must be encouraged since it is impossible to set up controlled studies under all possible conditions which might be encountered.

Along with the problem of controlled clinical trials of investigational new drugs is the problem of what to do with the animals which have been employed in the studies. The use of animals of economic importance may impose severe financial constraints on sponsors of clinical trials if all of the animals required for the studies or their products were not then allowed to be marketed. The Food and Drug Administration has established procedures for obtaining authorization to market edible products derived from investigational animals. These regulations primarily require data to show that consumption of edible products derived from animals treated with the highest recommended dosage with the minimum withdrawal time, will either not be inconsistent with the public health or does not contain drug residues or metabolites. If this can be shown then a series of procedures are available for obtaining authorization to market the edible products derived from the experimental animals. If this safety information is not available, the animals or their products cannot enter the food chain. This makes it necessary for the manufacturer to develop a great deal of toxicologic and metabolic information prior to undertaking extensive field trials.

I have outlined rather briefly some of the stages through which new chemicals are carried by toxicologists and clinicians. All of the data obtained in their endeavors must be compiled and submitted to the appropriate regulatory agency for evaluation. The toxicologists, pharmacologists, chemists and clinicians in the regulatory agency must then review all of this data and reach some conclusion about the probable safety and efficacy of the new agent in the field. This can be every bit as difficult a job as is generating the data initially and of course the impingement of data from a relatively large number of manufacturers onto a relatively small number of scientists in the regulatory agency may sometimes lead to delays in processing new drug applications or pesticide petitions. The thalidomide experience of 1962-1963 is a case in point where sitting on a new drug application probably prevented the wider spread dissemination of this new drug. I am certain that there might be instances where delay in release of a new chemical agent might cause some suffering or loss of life. One must weigh both probabilities and decide which course is less likely to produce serious consequences.
In summary, I would like to state that the toxicologic investigation of new chemical agents for use in medicine and agriculture is a very important part of the chemical and drug industries today. The veterinary profession has been playing an extensive role in the safety evaluation of drugs for both humans and animals. The most important point which must be kept in mind when working in this field is the impact that these new chemicals might have on the health of the public. Both industry and government are highly motivated in this direction and are to be commended for the success which their joint efforts have achieved.

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The problem of nitrate-nitrite poisoning characterized by methemoglobinemia has been known in this country for at least thirty years. Under natural conditions most cases occur in cattle, but occasionally it is seen in sheep and swine and rarely in horses. Sheep and horses are easily poisoned experimentally by nitrates but swine are poisoned only by nitrite as unlike cattle, sheep and horses they do not convert nitrate to nitrite. Sheep are less susceptible than cattle because of their greater ability to convert nitrite to ammonia in the rumen.

Outbreaks of nitrate-nitrite poisoning in cattle have been recorded following the ingestion of many different plant species in the growing, wilted or dried state and as silage. Some of the plants which have been incriminated are: the cereal grains especially green oats and oat hay (Avena sativa), Sudan grass (Sorghum sudanense), rye grass, (Lolium spp.), weeds such as redroot (Amaranthus retroflexus), mintweed (Salvia reflexa), variegated thistle (Silybum marianum), winged thistle (Cardus tenuifloris), and various vegetables, such as turnip tops, rape and kale (Brassica spp.) and mangolds (or mangels), (Beta vulgaris var. rapa). Under suitable conditions of moisture and temperature nitrate can be reduced to nitrite in cut plant material. This reduction is a stage in the conversion of nitrates to oxides of nitrogen.

There are various reasons for the accumulation of nitrate in an excessive amount in plants, but the use of artificial nitrogenous fertilisers, especially those having sodium, potassium or ammonium nitrate, is probably the most frequent cause. In plants nitrates are used to form protein and this may explain why the immature plant has less nitrate than the mature plant. When plants are grown in the shade or in drought conditions, and when they are wilted, either naturally or after being sprayed with weed-killers, they accumulate nitrate. It is known that the enzyme nitrate reductase needs sunlight to function, hence the difference in nitrate content when plants are grown in sunlight or in the shade.

Occasionally cattle have been poisoned by nitrates which have accumulated in well waters in toxic amounts and pigs have been poisoned by nitrite in whey following the use of nitrate to control undesired bacterial growth in the process of cheese-making. An outbreak of nitrite poisoning in pigs with very heavy losses following the feeding of cooked mangolds was reported in New Zealand. The cause was traced to the fact that in the cooking process a high enough temperature was not reached and conditions very suitable for the conversion of nitrate to nitrite existed.

In the rumen, nitrate is converted by micro-organisms to nitrite.
which is absorbed and converts hemoglobin to methemoglobin. This leads to generalised tissue hypoxia.

CLINICAL SIGNS

As with all the facets of nitrate-nitrite poisoning it is difficult to separate fact from fancy. The signs of acute nitrate poisoning are referable to hypoxia and possibly to the lowering of blood pressure and vascular dilation which are well known effects of nitrite. How rapid the course is from the initial signs to death or recovery, depends on many factors: the hemoglobin level of the particular animal, the amount of nitrate and/or nitrite ingested, the rate of conversion of nitrate to nitrite in the rumen, the rate of absorption of nitrite and conversion of hemoglobin to methemoglobin and the initial hemoglobin concentration. Thus the development of methemoglobinemia may be so rapid that a level incompatible with life is reached without any clinical signs being noted. When the conversion to methemoglobin is more gradual, the most noticeable sign is usually an apparent ataxia or more accurately staggering as the critical stage of hypoxia is approached. Laboured breathing and cyanosis, especially on exercise, may be observed. If cattle which have reached the staggering stage, are not treated promptly, they usually go down and soon die. However, the level of methemoglobin may not go beyond the fatal threshold which varies greatly from animal to animal and in the same animal from time to time. Usually it is in the range of 70-80 percent conversion of hemoglobin to methemoglobin. If it be possible to examine an affected animal, signs such as a rapid weak pulse, tachycardia, and a distinct brownness of the oral mucosa and the conjunctival membranes will be noted. Until relatively recently it has been considered that there was no such condition as chronic nitrate poisoning and there was good experimental evidence to show that cumulative poisoning did not occur. Within the past few years, however, there has been a growing volume of literature and opinion that the prolonged ingestion of nitrate could cause almost any clinical sign one could name. The two conditions which have received the most attention are abortion and hypovitaminosis A. Experimental studies on these aspects will be discussed later.

AUTOPSY FINDINGS

Many of the lesions reported in the literature for nitrate poisoning are nothing but figments of fertile imaginations or the idle prattle of persons who think that they must report something abnormal. Indeed the same can be said for many of the alleged lesions reported for almost any poison one cares to name. There are several reasons for this but the two principal ones are that: (1) many reports come from natural outbreaks and are made by persons inexpert in autopsy pathology; almost anything abnormal (and not all the "lesions" are abnormal) is considered to be caused by or associated with the suspected poison, and
there have been relatively few properly controlled experiments where the post-mortem findings have been compared between a dosed and a control group. If the lesions were but half as severe as those reported no animal could ever recover if the methemoglobin level was in the critical range for very long. The autopsy findings in nitrate poisoning are related to the discoloration of the blood, to vasodilation, and to anoxia. The blood invariably has a distinct brownish discoloration and because of this all organs and tissues are likewise discolored. One may find sub-epicardial and sub-endocardial hemorrhages, hypostatic pulmonary congestion, and other changes which must not be considered specific in any way for nitrite poisoning. We read of degenerative changes being found in parenchymatous organs, especially the liver and kidneys, on both gross and microscopic examination but there were no controls.

EXPERIMENTAL STUDIES IN NITRATE POISONING IN CATTLE

Bradley, Eppson and Beath showed conclusively that chronic nitrate poisoning did not occur when cows were dosed daily with a sub-lethal dose of potassium nitrate. This has been confirmed by Winter & Hokanson who fed 15 heifers sodium nitrate daily from the time they were two months pregnant until they calved or aborted. In the same experiment four heifers were fed sodium nitrate and four hydroxylamine hydrochloride. The methemoglobin was kept at 20-30 percent of total hemoglobin in one group and at 40-50 percent or higher in another. These experiments yielded irrefutable evidence that chronic nitrate-nitrite poisoning did not occur but that at any time, depending on various factors, the methemoglobin could rise to a fatal level. Moreover, although three heifers aborted there was no evidence of a causal relationship between the treatment and the abortions. Vibrio fetus was isolated from one fetus but nothing significant was found on bacteriologic or pathologic examinations of the others. Post-mortem examinations were done on nine heifers within ten days after parturition or abortion and on two heifers within two hours after death from methemoglobinemia. The only change seen macroscopically was a brownish discoloration of all organs and tissues. Microscopically it was reported that there was partial necrosis of renal glomeruli as well as minor changes in coronary arteries and the myocardium of some of the heifers but again there were no control tissues for comparison. There was no evidence, however, that these changes were directly related to the nitrate, nitrite or methemoglobin and an illustration of the glomerular lesion did not demonstrate any clear evidence of necrosis. Although these authors reported low serum vitamin A in heifers fed nitrate or nitrite, they were unable to establish a casual relationship. Later studies by Crawford, Kennedy and Davison, likewise failed to support the contention that nitrate causes abortion even when fed at a high level. In their experiments, heifers which were fed high nitrate hay, gained more
weight than the controls over a 35 day feeding period. If further evidence is needed to negate the ill-founded contention that nitrate causes abortion the paper from a group of workers at Cornell\textsuperscript{6} should be consulted. In a well controlled study in which nitrate was fed at fixed levels beginning before conception and at various stages of gestation and continued up to the time of parturition, they were unable to show any evidence of a direct relationship between nitrate and three abortions that occurred in the 45 heifers in the experiment. One heifer which collapsed twice with typical clinical signs of anoxia, had over 90 percent methemoglobin. She made a good recovery when nitrate was withdrawn for two days and went on to produce a normal calf 100 days later. No lesions were found in the aborted fetuses or placentae nor in calves and placentae from the normal births. Despite these reports, the myth of nitrate-induced abortion continues to enjoy many protagonists. The authors of these reports have not been able to do more than show that nitrate was in the diet of some cows which aborted or had to give all but fatal doses in experimental studies before they could induce abortion. Usually the nitrate was at a level of up to one percent of the diet. One group of investigators\textsuperscript{23} in a report of the lowland abortion syndrome in Wisconsin described various macroscopic and microscopic lesions in aborted fetuses and membranes and in heifers which aborted. However, the so-called lesions were either autolytic changes or normal features such as thickening of the pleura over the posterior parts of the pulmonary diaphragmatic lobes. Several illustrations of alleged lesions likewise failed to show significant changes. Although blood was taken once a week from the 12 heifers grazing a weedy pasture no significant concentration of methemoglobin was found in any of the samples. Yet, in the face of all these negative findings, it was suggested that high concentrations of nitrate or nitrite might be associated with the problem of lowland abortion. Later studies\textsuperscript{25} by the same group confirmed abortion in 17 heifers grazing weed infested pastures whereas abortion did not occur in 10 heifers grazing formerly weed infested pastures in the same area. No difference was found in the methemoglobin levels of the heifers that aborted and those that bore normal calves at full term. The weeds were said to contain "large amounts of nitrate" but no figures were reported. Likewise no results of bacteriologic, virologic, or serologic examinations were given. The aborted fetuses and membranes were said to have the same "lesions" as described previously. Although no cause for these abortions was found it was obvious that the authors were trying to incriminate nitrate. Subsequently they succeeded in inducing abortion experimentally by giving up to 140 gm. of $\text{KNO}_3$ daily directly into the rumen.\textsuperscript{24} Three heifers given 140 gm. died after the second dose; two given 100 gm. aborted after a few daily doses and in another given 100 gm. the fetus died and was retained. Again placental and fetal "lesions" were said to be the same as in lowland abortions, including the normal thickening of the pleura. None of three heifers given 70 gms. daily aborted. In the group in which the abortions occurred the methemoglobin apparently was
bordering on a fatal level as one heifer had to be given methylene blue. Experimental evidence such as this has been responsible for the fashionable diagnosis of 'nitrate abortion'. It has even been quoted by veterinary extension workers who have apparently chosen to ignore the well controlled experiments which lend no support to the theory that prolonged ingestion of sublethal amounts of nitrate causes cows to abort. Reports of failure to induce abortion in cows by the feeding of nitrate continue to appear. The most recent tells of pregnant dairy cows fed high nitrate silage over a nine week period. As in other experiments none of the pregnancies was interrupted. The situation is about the same in sheep. Setchell and Williams in Australia dosed pregnant ewes daily with nitrate during the last three months of pregnancy but no abortions occurred even though a fatal methemoglobin level was reached in some ewes. Similar results have been reported from the U.S.A. and Britain.

**EFFECTS OF NITRATE ON VITAMIN A**

There have been several poorly founded reports that nitrate in rations for cattle caused a lowering of the vitamin A reserves and because of them, feedlot cattle in particular, have been given larger and larger doses of vitamin A. Much of the evidence for this theory was extrapolated from experiments with rats and swine. Weichenthal studied the influence of sodium nitrate when fed at the rate of one percent of the total ration on performance and vitamin A of feedlot cattle. There was a marked depression of the rate of gain, which was not improved by 12,000 I.U. of vitamin A daily. The feed consumption was depressed about two pounds daily when NaNO₃ was fed but vitamin A and carotene in the plasma or liver were not affected.

It has been shown that whereas a daily level of 10,000 I.U. would not maintain initial vitamin A liver stores of 100 μg/gm of wet liver, 40,000 I.U. would. The plasma vitamin A values were found to be related to the amount of vitamin A in the diet rather than to the amount in the liver, hence analyses on plasma are of limited value.

More recent studies did not disclose any significant differences between nitrate dosed cows and control cows with respect to vitamin A, length of estrus cycles, birth weight of calves or milk production. Essentially the same findings have been reported by another group of workers who could find no effect of nitrate feeding on plasma carotene or vitamin A nor evidence of any adverse effects on pregnant lactating cows over a nine week period. It has also been shown that there is a dramatic erythropoietic response by heifers to prolonged feeding of nitrate. This compensatory mechanism is activated by the induced hypoxia and is sufficient to enable the blood to carry near normal amounts of oxygen in spite of high levels of methemoglobin. There was a marked increase in circulating red cells, hemoglobin concentration and blood volume in the heifers under test.
As with the other aspects of nitrate poisoning there is much confusion and contradiction in the literature concerning the amount of nitrate that is fatal. There are many and varied reasons for this and doubtless one of the most important is individual variation and susceptibility. Under some circumstances forages with 1.5 percent nitrate as KNO₃ on a dry matter basis may cause signs of toxicity and even death in cattle, depending on the total consumed and the rate of conversion to nitrite. Much higher levels of nitrate, up to five percent, can be eaten in forage with impunity, if the total is not too high.⁵,⁷ It was estimated by Dodd & Coup⁷ that a cow of about 700 pounds live weight could easily eat a fatal amount of nitrate during a grazing period of two hours. An animal that eats more quickly than another is thus more likely to succumb to nitrate poisoning. Therefore many factors must be considered, and it must be anticipated that a toxic level will vary from animal to animal.²⁹

**THE DIAGNOSIS OF NITRATE-NITRITE POSIONING**

In order to have any credence in a blood methemoglobin value, the blood must have been taken from a live animal or one that has been dead only a short time (about one to two hours) and must be examined within an hour or so. The spontaneous conversion of hemoglobin to methemoglobin in vitro and in vivo is the reason why the examination must be done as soon as possible. In air dried blood smears the conversion to methemoglobin is almost complete and immediate,¹³ but thick smears are useful for testing for nitrite by the diphenylamine test.¹³,¹⁴ Blood for spectrophotometric examination for methemoglobin should have potassium oxalate added as an anti-coagulant.

A very useful test for nitrate or nitrite can be done with a modified diphenylamine reagent and gives significant results up to 24 hours after death, on serum, plasma, fetal bile, urine and pleural, pericardial or peritoneal fluid. The procedure is extremely simple and consists of adding a few drops of reagent to one drop of body fluid. A positive reaction is shown by an immediate intense blue but is not specific for nitrate or nitrite as bromides, chlorates, selenites, molybdates, iron, antimony and peroxides also give the blue reaction. The history should help to eliminate or incriminate these substances. The experimental study showed that urine and pericardial fluid were most consistently positive. The authors suggested that several body fluids be tested and even when methemoglobin can no longer be detected, a positive diphenylamine test should warrant a tentative diagnosis of nitrate poisoning.

**DISCUSSION AND SUMMARY**

The classical form of nitrate-nitrite poisoning with methemoglobinemia is well known and recognised and need not be discussed further. At present, we are more concerned with various other effects which prolonged ingestion of sublethal amounts of nitrate is said to cause.
Although there did appear to be some justification for believing that nitrate-nitrite caused these previously unrecognised effects, the evidence was based mainly on clinical observation and the results of experiments with rats. It should be well known that one must be careful in transferring experimental findings from one species to another, especially when they are as far apart as the rat and the bovine animal. It should also be well known that clinical observation is not a substitute for experimental proof. When one is looking for a scapegoat it is not too difficult to find one, and nitrate seems to be the scapegoat in the problem of undiagnosed abortion in cattle. The several experimental studies reported herein, have established that occasionally a cow will abort if it has had sufficient nitrate-nitrite to produce a near fatal methemoglobinemia but they have not yielded any support for the other alleged effects.

I cannot give too strong a warning to take great care over the interpretation of what one sees at an autopsy and subsequently in tissue sections. The literature is replete with examples of "lesions" which were in fact normal findings or were in no way connected, except by chance, with the disease under investigation. All too often the gross and microscopic findings in treated or affected animals are not compared with the same in control animals. This is the very thing that has happened in some of the work reported here and regrettably it has been reported and quoted widely. One must be particularly wary of making false interpretation of possible fetal and placental lesions. The interpretation is doubly difficult if autolytic changes are present. This problem is by no means confined to veterinary pathology for it is a well recognised one in human pathology also.20

In summary then, there is no evidence from well controlled experiments, carried out in several different places by different groups of investigators to lend any support to claims that prolonged ingestion of sublethal amounts of nitrate causes abortion in cattle, nor any other ill effects such as a disturbance of vitamin A nutrition, decreased milk production, poor feedlot performance, lowered birth weight of calves and so on. The only effect which has consistently been shown in nitrate-nitrite poisoning is the conversion of hemoglobin to methemoglobin, which may or may not reach a fatal level, depending on a great many different factors.

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A DATA RETRIEVAL SYSTEM FOR VETERINARY DIAGNOSTIC LABORATORIES

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The recording of information dates back to many centuries B.C.; the ancient Egyptians used hieroglyphics to record the events of importance and this was done to provide someone in the future with insight into the past. The same problems existed with hieroglyphics as exist with much of our present day recorded data—it is nearly impossible to sort out the good information from the reams of data on file and present it in an informative manner.

Many methods including scrolls, microfilm, magnetic tape and magnetic disk have been used to record the past and man has used various machines from the abacus to present-day electronic computers to assist in calculations and analysis of data.

The computer is another tool which has many applications from routine statistical analysis of data to complex simulation of biological processes. I would like to visit with you about the use of a computer in regard to "A Data Retrieval System for Veterinary Diagnostic Laboratories."

Since the Veterinary Diagnostic Laboratory serves as a source of information to public health and regulatory personnel, educators, research scientists, practitioners and others, the need for a system which could provide these people with up-to-date accurate information was apparent.

A "Computerized System" was developed about a year ago which provided for the systematic recording and retrieval of administrative data as well as disease information.

In the planning of the system several aspects were considered:

1. The system should provide, at the end of each month, a complete analysis of the laboratory services performed such as gross and microscopic pathology, microbiology, serology, parasitology, chemistry and toxicology.
2. The data reported should reflect laboratory diagnoses of animal diseases with special emphasis on the epidemiological aspects.
3. Initiation and maintenance of the "Computerized System" should be done with a minimum of additional record keeping required.
4. The coding system should be efficient, flexible, and adaptable to a suitable "Standard Nomenclature."

The original data retrieval system has been in use about one year. Two aspects of the system have been changed. A new abstract sheet has been developed which will provide for more complete information regarding the accession and at the same time be easier for the diagnostician

*Dr. Hutton is an Instructor and Dr. Seaton is Professor and Head of the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa.
to complete. The coding system for recording diagnoses has also been changed. In the original data retrieval system a numeric coding system was developed and based on a categorical classification including species of animal, diagnoses, bacteriological findings, and condition of specimen such as tissue, alive, dead and culture or swab. Subdivisions under each of these categories were coded numerically starting with a code of 01 and continuing to 99 or 999 as the need arose. We are now using the Standard Nomenclature of Veterinary Diseases and Operations coding system. This

Veterinary Diagnostic Laboratory  
College of Veterinary Medicine  
Ames, Iowa

<table>
<thead>
<tr>
<th>Institution</th>
<th>Card No.</th>
<th>Accession Number</th>
<th>Accession Date</th>
<th>County or Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Month</td>
<td>Day</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Veterinarian or Agency</th>
<th>Species or Specimen</th>
<th>Number of Specimens (animals)</th>
<th>Condition of Specimen</th>
<th>Bread of Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Alive</td>
<td>5</td>
<td>Non-animal</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Dead</td>
<td>6</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Tissue</td>
<td>7</td>
<td>Swab</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Fecal</td>
<td>8</td>
<td>Other (Specify)</td>
</tr>
</tbody>
</table>

| 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 |

<table>
<thead>
<tr>
<th>Sex:</th>
<th>Age:</th>
<th>Delivered by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>34</td>
<td>35</td>
</tr>
</tbody>
</table>

| 0 | Unknown | 4 | Female (spayed) | 0 | Unknown | 5 | 1 - 2 yrs. | 1 | Male |
| 1 | Litter  | 5 | Male (castrate) | 1 | 0-2 wks. | 6 | 2 - 7 yrs. | 2 | Highway Patrol |
| 2 | Female  | 6 | Female (unknown) | 2 | 2 wks. - 2 mos. | 7 | 4 - 7 yrs. | 3 | Other |
| 3 | Male    | 7 | Male (unknown)  | 3 | 2 - 6 mos. | 8 | 7 - 10 yrs. | 4 | Over 10 yrs. |
| 8 | Other   | 4 | 4 - 6 - 12 mos. | 9 | Over 10 yrs. | 5 | Over 10 yrs. | 6 | Over 10 yrs. |

<table>
<thead>
<tr>
<th>Number of Animals in Affected Group</th>
<th>Morbidity</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 37 38 39</td>
<td>40 61 62 43 44 45</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Morbidity and Mortality</th>
<th>Rabies</th>
<th>Human Exposure</th>
<th>F. A. Exam</th>
<th>Animal Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Diagnosis</td>
<td>49 50 51 52</td>
<td>Yes 1</td>
<td>Pos. 1</td>
<td>Pos. 1</td>
</tr>
<tr>
<td>Second Diagnosis</td>
<td>53 54 55 56 57</td>
<td>No 2</td>
<td>Neg. 2</td>
<td>Neg. 2</td>
</tr>
<tr>
<td>Third Diagnosis</td>
<td>67 68 69 70 71 72 73 74 75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Number of Type 1 cards for this accession | 76 |
| Number of Type 2 cards for this accession | 77 |
| Card Type | 1 80 |

Figure 1
A DATA RETRIEVAL SYSTEM

system is similar to the one used previously and will allow the user to add diagnoses or findings as the need arises. It also provides for an exchange of information between Veterinary Diagnostic Laboratories or other institutions which are using the same coding system.

In the development of a retrieval system one of the first steps is to develop an abstract sheet (Figure 1) which will provide for the systematic recording of the data. The abstract sheet was developed to be filled out in English by the diagnostician and coded numerically by the secretary. This sheet is in two parts. The first part is for recording data such as

<table>
<thead>
<tr>
<th>Institution</th>
<th>Card No.</th>
<th>Accession Number</th>
<th>No. Post Mortem Examinations</th>
<th>Chemical &amp; Toxicological Examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Inoculation Examinations (Other than Rabies)</th>
<th>Fluorescent Antibody Examinations (Other than Rabies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Species Used</td>
<td>Number of Tissues Examined</td>
</tr>
<tr>
<td>1. Guinea Pig</td>
<td>1. Hog cholera</td>
</tr>
<tr>
<td>2. Rabbit</td>
<td>1. Positive</td>
</tr>
<tr>
<td>3. Mice</td>
<td>2. Negative</td>
</tr>
</tbody>
</table>

Laboratory Services Performed on this Accession

<table>
<thead>
<tr>
<th>Bacteriology</th>
<th>Sensitivity</th>
<th>Virus Isolation Attempted</th>
<th>Parasitology</th>
<th>Mycology</th>
<th>Hematology</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. yes</td>
<td>1. yes</td>
<td>1. yes</td>
<td>1. yes</td>
<td>1. yes</td>
<td>1. yes</td>
</tr>
<tr>
<td></td>
<td>2. no</td>
<td>2. no</td>
<td>2. no</td>
<td>2. no</td>
<td>2. no</td>
<td>2. no</td>
</tr>
</tbody>
</table>

Laboratory Findings (Bacteriology, Parasitology, Mycology, Virology, Chemistry & Toxicology)

<table>
<thead>
<tr>
<th>Serological Test Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lepto</td>
</tr>
<tr>
<td>2. Brucellosis</td>
</tr>
<tr>
<td>3. Anaplasmosis</td>
</tr>
<tr>
<td>4. IBR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histology, Field Trip, Kodachrome, Preliminary Report, Card Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Slides Lab. No.</td>
</tr>
<tr>
<td>65 66 67 68 69 70 71 72</td>
</tr>
</tbody>
</table>
species of animal, number of animals, condition of the specimen, age, sex, breed and morbidity and mortality information. Space is also provided for the recording of three diagnoses on each accession, however, through use of trailer cards any additional diagnoses may also be recorded. The second part of the abstract sheet (Figure 2) is used to record laboratory services performed on the accession as well as the laboratory findings. The number of serological tests performed can be recorded as well as the results of these tests. The number of histopathologic slides prepared and the serial number of these slides is recorded. This information is recorded so that if an investigator wishes to study the histopathological aspects of a certain disease, he can be provided with a list of the serial numbers of the slides that correspond to the diagnosis in which he is interested. Spaces were reserved to provide information regarding the number of days between the accession of a case and the preliminary report and final report sent to the veterinarian. This can provide insight into the length of time it takes to process various types of cases and can give perspective regarding areas of the laboratory where more personnel or equipment may be needed. The information regarding the number and type of laboratory services performed can provide concrete information when substantiating budget requests.

Upon termination of the laboratory procedures the diagnostician completes the case abstract and gives the abstract to the secretary for coding. Monthly the coded abstracts are forwarded to the Iowa State University Computation Center for processing and subsequent printing of monthly reports. The reports and case abstracts are returned to the diagnostic laboratory and the punched card file is maintained at the computation center as an information bank for further referral. Based on experience with approximately 5,000 cases per year, the time spent by the diagnosticians in filling out the forms is about two or three minutes per case.

Table I is a sample of information printed at the end of each reporting period. The number of specimens received by species as well as the quantity of the various laboratory procedures performed are a part of the monthly report. A listing is also produced (Table II) which shows by species the various diagnoses. Table III is also part of the computer output and gives a graphic representation of where the cases are originating. This can provide a ready source of information regarding enzootic diseases and is valuable in studying human and animal health. These reports aid in disease surveillance, epidemiological studies, and research and also give perspective on the use of technical personnel, work load, and anticipated expendable supply and equipment needs of the laboratory.

No attempt has been made to present the technical aspects of the computer programming required, however, the authors may be contacted in this regard. The programming has been done in PL/1 language and IBM System/360 Model 50 is the computer used for the processing of data. The storage capabilities of the computer allow for output of the English equivalent of the codes used for the various categories of information.
The Veterinary Diagnostic Laboratories are a tremendous storage bank of information and can be a great asset to those interested in animal diseases. The diagnostic laboratory may well be the "First Line of Defense" in some of our future disease problems. With the diagnostic laboratory we are dealing with field problems and obtaining a laboratory diagnosis. The disease problems that come in are because the veterinarian needs assistance in diagnosing the condition and it may be here that

**TABLE I**

**VETERINARY DIAGNOSTIC LABORATORY**

**IOWA STATE UNIVERSITY**

**AMES, IOWA**

**MONTHLY REPORT**

**July 1966**

**LABORATORY SERVICES**

The following refer to number of individual animals or specimens examined

| Total specimens entered other than serology | 733 |
| Post mortem examinations                    | 237 |
| Bacteriological examinations                | 415 |
| Chemical and Toxicological analyses         | 222 |
| Parasitological examinations                | 27  |
| Rabies FA examinations                      | 192 |
| Meat inspection                            | 40  |

**SEROLOGY**

| Leptospirosis Agglutination Tests          |
|                                          |
| Specimen                                |
| Bovine                                  |
| Total specimens entered                 | 1149 |
| Negative                                | 1095 |
| Positive                                | 54   |
| Porcine                                 |
| Total specimens entered                 | 2623 |
| Negative                                | 2613 |
| Positive                                | 10   |

| Anaplasmosis Capillary Agglutination Tests |
|                                          |
| Specimen                                |
| Bovine                                  |
| Total specimens entered                 | 25   |
| Negative                                | 23   |
| Positive                                | 2    |

| IBR Serology                            |
|                                          |
| Specimen                                |
| Bovine                                  |
| Total specimens entered                 | 33   |
| Negative                                | 23   |
| Positive                                | 10   |

| RABIES                                   |
|                                          |
| Specimen                                |
| Bovine                                  |
| Total specimens entered                 | 12   |
| Negative                                | 11   |
| Positive                                | 1    |
| Canine                                  |
| Total specimens entered                 | 30   |
| Negative                                | 30   |
| Positive                                | 0    |
| Feline                                  |
| Total specimens entered                 | 45   |
| Negative                                | 42   |
| Positive                                | 3    |
| Skunk                                   |
| Total specimens entered                 | 10   |
| Negative                                | 5    |
| Positive                                | 5    |
| All other species                       |
| Total specimens entered                 | 95   |
| Negative                                | 94   |
| Positive                                | 1    |

**Total animal examination, by species, during report period; serological tests not included.**

<p>| Bovine | 119 |
| Canine | 99  |
| Feline | 51  |
| Porcine| 178 |
| Poultry| 110 |
| Skunk  | 10  |
| All other species| 206 |</p>
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Animals</th>
<th>Cases</th>
<th>Diagnosis</th>
<th>Animals</th>
<th>Cases</th>
</tr>
</thead>
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<tr>
<td><strong>BOVINE</strong>: Total 119</td>
<td></td>
<td></td>
<td><strong>POULTRY</strong>: Total 110</td>
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<td>Abortion</td>
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<td>Arthritis/Staph</td>
<td>4</td>
<td>2</td>
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<td>Anemia</td>
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<td>1</td>
<td>Aspergillosis</td>
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<td>1</td>
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<tr>
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<td>2</td>
<td>1</td>
<td>Blue comb</td>
<td>3</td>
<td>1</td>
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<tr>
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<td>1</td>
<td>Coccioidosis</td>
<td>8</td>
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<tr>
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<td>2</td>
<td>Inanition</td>
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<td>Leucosis/neutral</td>
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</tr>
<tr>
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<td>1</td>
<td>Leucosis/visceral</td>
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<td>1</td>
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<td></td>
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<td></td>
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<tr>
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<td>1</td>
<td>Ascarids</td>
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<td>Capillaria sp.</td>
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<td>Synovitis</td>
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<td>2</td>
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<td>1</td>
<td>Pancreatitis</td>
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<tr>
<td>Arthritis/suppurative</td>
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<td>1</td>
<td>Bacteriological exams.</td>
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<td>3</td>
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<td>1</td>
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<td>3</td>
<td>Bacteriological exams.</td>
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<td>1</td>
<td>Feline:</td>
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<tr>
<td>Gastric ulcer</td>
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<td>1</td>
<td>Arsenic poisoning</td>
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</tr>
<tr>
<td>Heat prostration</td>
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<td>1</td>
<td>Distemper</td>
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</tr>
<tr>
<td>Hog cholera</td>
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<td></td>
<td>Neoplasm:</td>
<td></td>
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<td>1</td>
<td>Gerbil:</td>
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<td>Heat prostration</td>
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<td>Pheasant:</td>
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<td>Inanition</td>
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<td>Myoclonia congenita</td>
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<td>Pigeon:</td>
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<td>1</td>
<td>Botulism</td>
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<td>Rabbit:</td>
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<tr>
<td>Peritonitis</td>
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<td>Pasteurellosis</td>
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</tr>
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<td>Pneumonia/ unspecified</td>
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<td>Staphylococci</td>
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<td>Pneumonia/ unspecified</td>
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</tr>
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<td>1</td>
<td>Squirrel:</td>
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<tr>
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</tr>
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<td>5</td>
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<td>Tuberculosis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VPP/Positive</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteriological exams.</td>
<td>34</td>
<td>19</td>
<td></td>
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</tbody>
</table>
new diseases are uncovered. It may be possible through a central reporting system that pesticide problems could be brought to the attention of the proper personnel readily, low incidence diseases may be seen, and foci of epizootics could be detected early. The data obtained from Veterinary Diagnostic Laboratories along with other similar information can give perspective regarding morbidity and mortality information and the economic loss which results from animal diseases.

REFERENCES


THE SPICER-EDWARDS TECHNIQUE AND ITS APPLICATION IN SALMONELLA IDENTIFICATION
E. M. Ellis, D.V.M., Ph.D.*
Ames, Iowa

With the ever-increasing interest in Salmonella contamination in animal feeds and Salmonellosis as a disease of animals and of man, a tremendous burden has been placed on laboratories for the serotyping of Salmonella isolants. It is our purpose to introduce into the routine of as many diagnostic laboratories as possible a Salmonella screening procedure known as the Spicer-Edwards technique.

The earliest scheme for the simplified typing of Salmonellae was that of Kaufmann in 1930.1 Other simplifications have appeared, particularly those of Kauffmann and Edwards (1947)2 and Edwards and Kauffmann (1952).3 Working from this scheme Spicer (1956)4 devised a system of four polyvalent additional antisera for identifying more than 17 H antigens. Dr. P. R. Edwards modified the method of Spicer by removing the L complex from the four pools and used it independently. He also rearranged the various antisera so that no single H antigen reacted with all four Spicer pools. The composition of the Spicer pools is illustrated in Table I.

We are all aware that any procedure short of specific serotyping must be considered a presumptive identification and not exact. We will try to point out the extent of the presumption when one uses the Spicer-Edwards technique.

<table>
<thead>
<tr>
<th>Polyvalent Serum Pool #1</th>
<th>Polyvalent Serum Pool #2</th>
<th>Polyvalent Serum Pool #3</th>
<th>Polyvalent Serum Pool #4</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>b</td>
<td>b</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>c</td>
<td>c</td>
<td>e,h</td>
<td>G</td>
</tr>
<tr>
<td>d</td>
<td>k</td>
<td>k</td>
<td>k</td>
</tr>
<tr>
<td>e,h</td>
<td>r</td>
<td>z</td>
<td>r</td>
</tr>
<tr>
<td>G</td>
<td>y</td>
<td>(Z_4)</td>
<td>z</td>
</tr>
<tr>
<td>i</td>
<td>z_{29}</td>
<td>z_{29}</td>
<td>z_{10}</td>
</tr>
</tbody>
</table>

G complex \((f,g,f,g,t; g,m; g,m,s; g,m,t; g,p; g,p,u; g,q; g,s,t; m,t; etc.)\)
Z\(_4\) complex \((z_{4}, z_{23}; z_{4}, z_{24}; z_{4}, z_{32})\)
Individual H Sera
L complex \((1,v; 1,w; 1,z_{13}; 1,z_{28})\)
e,n complex \((e,n,x; e,n,z_{15})\)
1 complex \((1,2; 1,5; 1,6; 1,7; z_{6})\)

*United States Department of Agriculture, Animal Health Division, Diagnostic Services, National Animal Disease Laboratory.

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technique for screening Salmonella. A practical application on the use of the Spicer-Edwards technique is demonstrated by one diagnostic laboratory using the Spicer-Edwards technique as the basis for making a presumptive identification of Salmonella serotypes as follows:

The total number of serotypes reported was 297. These were specified using the Spicer-Edwards technique. When serotyped by NADL, 255 were in agreement with the serotype as reported by the typing center at NADL. It should be remembered that the cultures came from a small geographic area and the prevailing serotypes for that area were well known. The following are some of the serotypes found: S. chester, S. heidelberg, S. typhimurium, S. blockley, S. saint-paul, S. senftenberg, and eleven other serotypes seen less frequently.

Thirty-seven isolants were pinpointed by the Spicer-Edwards test as one of two possibilities. Only six isolants were serotyped by the reference center that were surprises or in disagreement with the presumptive identification of the submitting laboratory.

The number of recognized Salmonella serotypes today approximates 1000, and the complexity of O and H antigens described in the Kauffmann-White scheme lead many persons to feel that Salmonella identification should be done only in highly specialized centers. This is, however, not necessarily the case.

Of the 1000 known serotypes of Salmonella, the serotyping laboratory at NADL sees less than 10 percent. Therefore, the job of typing 100 different serotypes is less laborious than one would anticipate.

Phase identification is most important in the use of the Spicer pools. A word regarding this is in order. When one isolates a Salmonella, almost in every case, the organism will exist in one phase or the other. This is to say that, if two phases exist for that serotype, only one will be present or dominant in the culture.

Therefore, when the organism is placed in the Spicer pools, (as for example, S. typhimurium) (Table I) the i H antigen will be identified in pool #1. To determine the nondominant or second phase, the Spicer pools may be used. With S. typhimurium, the 1,2 phase may be identified by placing some of Spicer pool 1 serum in a semi-solid media. Pool 1 contains the i antiserum along with other serums such as a, b, c, d, e, h, and G (Table I). The presence of the additional H antibodies does no harm, and the i antibody present in pool 1 will tie up the i flagella allowing the detection of only the 1,2 flagella. Single factor serums are therefore not necessary for phase reversal, those included in the Spicer pools being adequate for this purpose. The organism in phase i is inoculated into the edge of a plate of semi-solid media containing i antibody. The organism is allowed to grow across the plate and harvested from the opposite side. These organisms are the ones in which phase 2 antigens predominate. Re-inoculating Spicer pools will reveal the phase 2 antigen.

The reaction of the Spicer pools can be seen in Table II. It should be obvious that, reading across the table, for each H antigen a through Z29, there are no two reaction combinations alike. Therefore if one had, as in the previous example, S. typhimurium, in tube number 1, agglutination
TABLE II
Reactions of H Antigens in Spicer Polyvalent Sera (Modified)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Polyvalent Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>a</td>
<td>+</td>
</tr>
<tr>
<td>b</td>
<td>+</td>
</tr>
<tr>
<td>c</td>
<td>+</td>
</tr>
<tr>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>e, h</td>
<td>+</td>
</tr>
<tr>
<td>G complex</td>
<td>+</td>
</tr>
<tr>
<td>i</td>
<td>+</td>
</tr>
<tr>
<td>k</td>
<td>-</td>
</tr>
<tr>
<td>r</td>
<td>-</td>
</tr>
<tr>
<td>y</td>
<td>-</td>
</tr>
<tr>
<td>z</td>
<td>-</td>
</tr>
<tr>
<td>z₄</td>
<td>-</td>
</tr>
<tr>
<td>z₁₀</td>
<td>-</td>
</tr>
<tr>
<td>z₂₉</td>
<td>-</td>
</tr>
</tbody>
</table>

would be visible while in tubes 2, 3, and 4 the antigen would not be agglutinated. One would then reverse the phase using the technique already described and repeat the agglutinations. The tube containing the 1 complex would be positive for agglutination (see Table I) while the tubes containing the L and en complexes would be negative.

It will be helpful to go back to the original culture of *S. typhimurium* and go through the procedure in logical sequence to see how a culture is examined using the Spicer-Edwards technique. The freshly isolated culture is almost always motile enough to use without passing it through motility media. Cultures that have been stored for a time become non-motile. A tube of heart infusion broth is inoculated at the same time the biochemical tests are set up. This culture is the source of antigen. After growth for 18-20 hours, the culture is treated with an equal volume of formalinized physiologic saline solution. While this is standing, the "O" group of the Salmonella can be determined using the five polyvalent O antisera, Groups B, C₁, C₂, D, and E in a slide agglutination test. In practice, other "O" serums are generally not necessary. In the case of *S. typhimurium*, the cultures will agglutinate in "O" group B. (Table III).

### TABLE III

<table>
<thead>
<tr>
<th>&quot;O&quot; Group B</th>
<th>Group B O Antigens</th>
<th>H Antigens</th>
<th>Spicer Pools 1 Complex</th>
<th>L Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>S. typhimurium</em></td>
<td>1, 4, 5, 12</td>
<td>1, 2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. <em>S. typhimurium</em></td>
<td>1, 4, 12</td>
<td>1, 2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>var. copenhagen</td>
<td>1, 4, 12</td>
<td>1, 2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. <em>S. agama</em></td>
<td>4, 12</td>
<td>1, 6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. <em>S. gloucester</em></td>
<td>1, 4, 12</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Returning to the formalinized culture, pipet 0.5 ml into each of 7 Kahn serologic tubes. Then add 0.5 ml of the required Spicer pools; 1, 2, 3, 4, L, en, and 1, each into a separate tube of antigen. Incubate the tests for one hour at 50°C and read for the presence or absence of agglutination. In the case again of *S. typhimurium*, Spicer tube I antigen will be agglutinated; antigen in tubes 2, 3, and 4 will not be agglutinated; additional tubes containing the L, en, and i antiserums will be negative. After reversing the phase of the culture as outlined previously, the I complex antiserum will agglutinate the antigen.

Referring to the Kauffmann-White scheme, (Table III) one can see that the slide agglutination test using polyvalent "O" serum indicated a group B organism. Spicer pools indicated an organism agglutinated by Spicer pool 1 indicating H antigen i, phase 1, and the 1 complex, phase 2. The only serotypes found in the Kauffmann-White scheme that have this antigenic formula indicated in "O" group B are seen in Table III.

It is our recommendation that the Spicer-Edwards technique can be used as a working, practical tool by local diagnostic laboratories. They can utilize a Salmonella Reference Center for confirmation purposes. It will provide the laboratories with immediate information about the Salmonellae with which they are dealing and this information can be useful in epidemiologic investigations.

This fact is made clear by Dr. P. R. Edwards in his publication, "Serologic Examination of Salmonella Cultures for Epidemiologic Purposes." The technique is routinely used at the Communicable Disease Center by Mrs. Mildred Galton, Chief, Veterinary Public Health Laboratory, Department of Health, Education and Welfare, Communicable Disease Center, Atlanta, Georgia, and is regarded as a most valuable screening device.

When information from the Spicer technique is sent to the serotyping laboratory along with the culture for exact identification, only a fraction of the usual time will be required to identify the serotype. With the cost of serotyping high and the demand for this service increasing daily, we should all investigate the possibility of using this valuable tool.

REFERENCES

PORCINE ENTERITIS DUE TO CLOSTRIDIUM PERFRINGENS TYPE C

I. EPIZOOTIOLOGY AND DIAGNOSIS


INTRODUCTION

Enteritis due to Clostridium perfringens type C infection was recognized as a distinct disease entity of nursing pigs in England\textsuperscript{3} and Hungary\textsuperscript{7,8} during the past decade. It was recently reported from Denmark.\textsuperscript{6} In the United States, this enteric syndrome was first reported in calves\textsuperscript{4} and lambs\textsuperscript{5} in Colorado. An epizootic of the disease in newborn pigs was encountered in Minnesota in 1963. Additional spontaneous epizootics in Minnesota and Iowa swine have been described.\textsuperscript{2}

EPIZOOTIOLOGIC OBSERVATIONS

Since the first case in Minnesota was recognized in 1963, specimens from 40 additional herds have been received at the University of Minnesota Veterinary Diagnostic Laboratory during 1964 to 1966. All affected herds were located in southern Minnesota and Iowa. Twenty-six of these herds were in a single 12 x 18 mile area of west-central Iowa (Figure 1).

Morbidity and mortality data were obtained from 33 of the affected herds. The data from 20 selected herds are given in Table I. In these 20 herds, the infection did not appear to be complicated by other diseases, and Cl. perfringens type C antitoxin was not administered during the course of the epizootics. Various antibiotic preparations were given to some litters in most of the herds, which may have altered the course of the disease and slightly reduced the case fatality rate.

There was no common source of feed ingredients, nor had there been direct contact between the swine and other livestock in the majority of the epizootics investigated. Two purebred Duroc-Jersey, two purebred Hampshire, and 16 crossbred herds were affected. One of the epizootics occurred in a specific-pathogen-free herd. There was no history of a similar disease syndrome in calves or lambs on any of the farms.

The sows had access to natural or man-made waterways prior to farrowing on three of the 20 farms. The disease reoccurred in the subsequent farrowing period in four herds. The probability of reoccurrence of the disease in the majority of the herds undoubtedly was greatly reduced

From the Department of Veterinary Diagnostic Laboratories (Bergeland) and Department of Veterinary Medicine (Sorensen), University of Minnesota, St. Paul, Minnesota, Dr. Dermody is a general practitioner at Breda, Iowa.

This study was supported in part by funds received from the Minnesota Agricultural Department Station and North Central Regional Research funds.
because the sows on most farms were vaccinated with *Clostridium perfringens* type C toxoid before the next farrowing. Wooden farrowing crates had been transferred among three of these farms and two boars had also been jointly used by the same three farmers. There were recent introductions of new breeding stock, either boars or gilts, in all herds prior to the onset of the disease.

**DIAGNOSIS**

The diagnosis of *Clostridium perfringens* type C enteritis was based on clinical and pathologic findings, demonstration of *Clostridium perfringens* type C toxin in intestinal content, and by isolation of type C *Clostridium perfringens* from scrapings of the intestinal mucosa.

**Clinical Observations**

The disease occurred in piglets ranging in age from one day to one month. In one herd, the disease first appeared in two to three week old pigs, however, the majority of the epizootics began in pigs less than one week old. The pigs commonly sickened late in the first day, or on the second day after birth. The heaviest mortality occurred on the second
TABLE I
Litter Morbidity and Piglet Mortality of 20 Epizootics of *Cl. perfringens* - C Enteritis

<table>
<thead>
<tr>
<th>Farm No.</th>
<th>No. Litters Farrowed</th>
<th>Affected Litters No.</th>
<th>Affected Litters Percent</th>
<th>No. Viable Newborn</th>
<th>Mortality* No.</th>
<th>Mortality Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>60</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>8</td>
<td>19</td>
<td>350</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>9</td>
<td>100</td>
<td>65</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>13</td>
<td>76</td>
<td>162</td>
<td>70</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>2</td>
<td>9</td>
<td>190</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>14</td>
<td>61</td>
<td>190</td>
<td>60</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>8</td>
<td>80</td>
<td>70</td>
<td>36</td>
<td>51</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>11</td>
<td>33</td>
<td>270</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>2</td>
<td>13</td>
<td>95</td>
<td>12</td>
<td>13</td>
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<td>10</td>
<td>23</td>
<td>15</td>
<td>65</td>
<td>214</td>
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<td>11</td>
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<td>21</td>
<td>106</td>
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<td>20</td>
<td>18</td>
<td>90</td>
<td>180</td>
<td>85</td>
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<td>2</td>
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<td>139</td>
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<td>6</td>
</tr>
<tr>
<td>14</td>
<td>17</td>
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<td>29</td>
<td>153</td>
<td>8</td>
<td>5</td>
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<td>18</td>
<td>18</td>
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<td>140</td>
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<td>57</td>
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<tr>
<td>16</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>70</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>12</td>
<td>67</td>
<td>160</td>
<td>35</td>
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<tr>
<td>18</td>
<td>25</td>
<td>25</td>
<td>100</td>
<td>240</td>
<td>115</td>
<td>48</td>
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<td>19</td>
<td>37</td>
<td>27</td>
<td>73</td>
<td>350</td>
<td>120</td>
<td>34</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>6</td>
<td>67</td>
<td>59</td>
<td>35</td>
<td>59</td>
</tr>
</tbody>
</table>

Total 397 208 - 3,263 863 -
Range 8-42 - 9-100 59-350 - 5-59
Average 19.9 10.4 52.4 163.2 43.2 26.4

*The mortality given is the number of pigs which were assumed to have died of *Cl. perfringens* - C enteritis, based on clinical observation, plus selected necropsies and laboratory examinations. The total herd mortality was slightly greater.

through the fifth days. The morbidity within litters was variable, ranging from a single pig per litter to the entire litter. Most commonly, only a portion of the affected litters sickened.

Diarrhea was consistently observed except for occasional peracute cases which suddenly collapsed and died before the onset of diarrhea. The color and consistency of the feces varied with the clinical course of the disease.

In the acute cases, which usually died late on the first or on the second postnatal day, bright red watery feces were evident. Cases with a subacute clinical course of two to three days had reddish brown liquid feces, whereas cases of slightly longer duration had colorless liquid feces in which particles of gray necrotic debris were suspended. Chronic cases often had pasty, gray feces.

The clinical course of the disease varied considerably in different herds, as well as among litters or within litters of a single herd. Acute cases manifesting a hemorrhagic diarrhea were seen in only seven of 20
epizootics investigated. More commonly, the syndrome was a fatal, non-hemorrhagic diarrheal disease of several days duration. Recovered cases were rare.

Pathologic Findings

The most acute cases were characterized by a necrohemorrhagic jejunitis. The serosal surface of the small intestine was dark red, and the lumen content was a bright red fluid. The ileum and colon were also filled with blood, however mucosal necrosis was usually confined to the jejunum. Masses of robust bacilli covered the necrotic jejunal villi, and there was extensive hemorrhage in the tunica submucosa and tunica muscularis. The mesenteric lymphatics and the sinuses of the mesenteric lymph nodes were engorged with blood. There was commonly an accumulation of sanguinous fluid in the peritoneal cavity.

Subacute cases with a clinical course of two to three days were characterized by emphysema of the wall of a sharply demarcated segment of the jejunum. The affected segment of the jejunum was usually loosely adhered by a localized fibrinous peritonitis. The mucosal surface was covered by yellow necrotic debris.

More chronic cases had varying degrees of mucosal necrosis of the jejunum and/or ileum. The mucosa of the affected segment often was completely replaced by bacteria, inflammatory cells, and cellular debris. In one pig examined, only a two cm. segment of ileum was affected.

Demonstration of Cl. perfringens Type C Toxin in Intestinal Content

Demonstration of toxin was accomplished only in acute cases which were examined shortly after death or which were frozen soon after death prior to shipment to the laboratory. The intestinal content was centrifuged and 0.25 ml. of the supernatant fluid was injected intravenously into mice. If death occurred within one hour, standard mouse protection tests using type-specific antiserum* were performed. Most of the cases were not the acute form of the disease, and there usually was some post mortem decomposition, therefore this procedure did not prove to be of value in the majority of the laboratory examinations.

Isolation of Cl. perfringens Type C from the Intestine

Type C Cl. perfringens was isolated from the intestine of all cases of the disease examined at the laboratory. The isolation was accomplished by direct streaking of scrapings of the intestinal mucosa on five percent sheep blood agar plates followed by incubation in an anaerobe jar for 18 to 24 hours. Two plates were inoculated from each intestine, one from the mid-jejunum and one from the lower ileum. Two or three isolated colonies were selected on the basis of colonial morphology, and each were transferred to a tube of chopped meat broth. The broth cultures were then incubated for six to nine hours and the culture supernatant fluid was tested for type C toxin by mouse protection tests.

*Obtained from Burroughs Wellcome and Company.
TABLE II
Types of *Clostridium perfringens* Isolated From the Intestine of Pigs One to 15 Days Old

<table>
<thead>
<tr>
<th>Colonial Types</th>
<th>Group 1*</th>
<th>Group 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Herds</td>
<td>No. Pigs Examined</td>
<td>No. Pigs <em>Clostridium perfringens</em> isolated</td>
</tr>
<tr>
<td>A</td>
<td>C</td>
<td>Total</td>
</tr>
<tr>
<td>----------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>41</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>53</td>
<td>97</td>
<td>58</td>
</tr>
</tbody>
</table>

*Cases of *Clostridium perfringens* - C enteritis.

**Piglets affected with other diseases, including *Escherichia coli* enteric disease, *Streptococcus* and *E. coli* umbilical infections, transmissible gastroenteritis, and viral polioencephalomyelitis.

The results of the bacteriologic examination of 156 pigs by this method are presented in Table II. Type C *Clostridium perfringens* was isolated from all pigs which were considered to have died from *Clostridium perfringens* Type C enteritis. *Clostridium perfringens* was isolated from only 58 of 97 piglets affected with other diseases, and all of the colonies typed from this group proved to be type A.

In our experience, the isolation of type C *Clostridium perfringens* was the single most useful procedure for the laboratory confirmation of the diagnosis, particularly in cases where post mortem decomposition prevented histologic evaluation of the specimen.

SUMMARY

*Clostridium perfringens* type C enteritis of nursing pigs was diagnosed in 41 herds from southern Minnesota and Iowa during 1963 to 1966. In 20 herds where accurate data were available, approximately 50 percent of the total litters were affected, and the average herd mortality was approximately 25 percent. Diarrhea was common to all of the epizootics, and hemorrhagic diarrhea was seen in one-third of the herds. The disease affected pigs one day to one month old, however the majority of the cases occurred during the first postnatal week.

The diagnosis was based on clinical findings, gross and microscopic intestinal lesions, and isolation of type C *Clostridium perfringens* from the intestinal mucosa.

REFERENCES


DERMAL LESIONS IN AVIAN LEUCOSIS  

Dermal leucosis, more commonly called skin leucosis, is believed by some workers to be one of the pathological manifestations of a group of viruses that are RIF and COFAL negative, and have a short incubation period. This group of viruses is believed to produce lesions of the nerves, viscera, muscle, skin, and eyes and has been variously classified under the term of Marek's disease or acute avian leucosis, \(^2\) "the neural, visceral and ocular forms of avian lymphomatosis,"\(^5\) and visceral lymphomatosis.\(^1\) Benton, \textit{et al.}\(^1\) state that skin lesions are closely aligned with visceral leucosis.

Much critical research remains to be done to isolate and adequately characterize this group of viruses and their relationship to the various lesions observed before a generally acceptable classification and nomenclature can be agreed upon. In the meantime, what current criteria are available to the laboratory diagnostician on which a reasonably accurate diagnosis can be based? At present the whole issue of diagnosis of the acute avian leucosis complex is confusing. But, gradually a pattern of association is evolving between the tumor producing agent(s), the observed lesions, and the clinical history.

This paper reviews data obtained from gross and microscopic examinations of broiler carcasses condemned in broiler processing plants in Arkansas between January and July, 1966.

DESCRIPTION

A cooperative agreement was reached between the Arkansas Poultry Federation, The Inspection Service, Poultry Division, United States Department of Agriculture, and the Department of Animal Sciences, University of Arkansas wherein a five percent random sample of condemned broiler carcasses was obtained and re-inspected by one of the authors (DHM) at the Veterinary Research Laboratory, University of Arkansas.

The broilers were processed between six and twelve weeks of age, were of mixed sex, and were managed essentially the same throughout the growing period and at processing. The carcasses in this study were obtained from nine different processing plants and represent a good cross section of management practices and disease conditions throughout the Arkansas poultry industry.

In tabulating the observed lesions in this laboratory, all carcasses exhibiting skin lesions of leucosis were classified in that category regardless of the presence of any other concurrent lesion in the same

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carcass. If visceral lesions of leucosis were observed, and the skin lesions were absent, they were classified in the "visceral" category regardless of other concurrent lesions present. Additional data were obtained late in the project when it became apparent that there was a higher incidence of the skin and visceral forms of leucosis in the females. Thereafter, observed lesions were tabulated according to sex.

In numerous instances several different condemnable conditions were observed in the same carcass and these were tabulated under "concurrent lesions."

Specimens were selected from among the condemned carcasses from time to time for additional gross and microscopic examination. Various tissues were obtained, but usually included brain, heart, liver, spleen, kidney, gonad, adrenal, sciatic nerve and skin from the leg, breast and neck. The tissues were fixed in 10 percent neutral formalin and stained with hematoxylin-eosin.

The carcasses were selected from among those exhibiting no observable gross lesions, skin lesions of leucosis only, visceral lesions of leucosis only, or both skin and visceral lesions.

**OBSERVATIONS**

**Gross**

The skin lesions of leucosis were found in the feather follicles where they exhibited varying degrees of nodular enlargement, were yellow-orange-to cream-or gray in color, had a glistening appearance and, in some instances, appeared hyperemic. The numbers of affected follicles varied from only a few to an extensive involvement of 100 or more. Tumorous discrete follicles commonly were scattered along the feather tracts of the leg, thigh, breast, cervical area and wings in descending order of occurrence and frequently were scattered irregularly between the feather tracts. The epidermis covering the affected follicles usually was retained intact, but in advanced cases, the adjoining tumorous masses appeared to coalesce, and sometimes produced general thickening and ulceration. Some strains of chickens normally have coarse prominent feather follicles which may be misleading to the inexperienced observer. When these follicles are examined critically they are normal in every respect except for size.

Some chickens exhibited numerous hyperemic follicles on the leg, thigh and rump. Such lesions may be due to feather picking or some toxic or septic syndrome. The hyperemic skin lesions of leucosis resembled these and added to the confusion of the differential diagnosis based on gross observations.

It is stated by some inspectors and processing plant personnel that skin lesions are more prominent immediately after scalding and depluming and if the carcasses have been chilled and then re-inspected the lesions are much more difficult to distinguish. A limited number of broiler carcasses classified as skin leucosis-positive in this laboratory were placed in individual plastic bags, covered with ice and held in a refrigerator
at 40°F for five days. Re-examination of the carcass, without reference to the previous classification, did not reveal any alterations in incidence or degree of involvement in any of the carcasses tested.

One problem that was encountered in this re-inspection project pertained to the difficulty in detection of skin lesions masked by the yellow "bloom" which appeared to be "fixed" by hard scalding. It was often necessary to remove this bloom by scrubbing with a brush.

Sixteen and one half percent of carcasses classified in this laboratory as skin leucosis exhibited concurrent visceral lesions. The observed lesions in the viscera consisted of miliary to large (15mm) discrete bulging white foci in the liver and spleen, enlargement and distortion of the kidney by white to gray tumorous masses throughout, and foliate enlargement of the ovary. The ovary usually had a translucent cream white appearance but occasionally was mildly hyperemic. Lesions in the testes ranged from small nodular enlargements to a massive U shaped distortion of the organ in advanced cases. The tumorous masses in the testes were cream-white to gray in color.

Lesions in the heart varied from small gray foci in the epicardium to massive gray translucent bulging masses that distorted the organ. On cut section the nodular masses often extended through the myocardium into the ventricles.

One and one half percent of the carcasses were classified under "bone leucosis." However, in no instance was osteopetrosis observed concurrently with skin or visceral lesions.

No gross lesions were observed in the lungs, nervous system, or eyes. Only a few lesions were observed in the skeletal muscle of the breast.

**TABLE I**
Broiler Condemnation Percentages by Lesions in 13,058 Birds

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airsacculitis</td>
<td>40.7</td>
</tr>
<tr>
<td>Leucosis - All Forms</td>
<td>42.9</td>
</tr>
<tr>
<td>Leucosis - Skin Lesions</td>
<td>39.7</td>
</tr>
<tr>
<td>Leucosis - Visceral Lesions</td>
<td>2.1</td>
</tr>
<tr>
<td>Leucosis - Bone</td>
<td>1.1</td>
</tr>
<tr>
<td>Septicemia - Toxemia</td>
<td>10.3</td>
</tr>
<tr>
<td>Other</td>
<td>6.1</td>
</tr>
</tbody>
</table>

**TABLE II**
Condemnable Lesions in Broilers by Sex

<table>
<thead>
<tr>
<th>Sex Distribution</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airsacculitis</td>
<td>53.3</td>
<td>29.0</td>
</tr>
<tr>
<td>Leucosis - All Forms</td>
<td>29.5</td>
<td>53.8</td>
</tr>
<tr>
<td>Leucosis - Skin Lesions</td>
<td>26.1</td>
<td>46.9</td>
</tr>
<tr>
<td>Leucosis - Visceral Lesions</td>
<td>1.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Leucosis - Skin Plus Visceral</td>
<td>27.9</td>
<td>53.2</td>
</tr>
</tbody>
</table>
Table II presents data on 2507 broiler carcasses and shows that there was a 53.2 percent incidence of skin and visceral lesions of leucosis among females and 27.9 percent incidence of these forms among males.

**MICROSCOPIC**

**Skin**

Focal masses of mononuclear, round cells commonly were observed in the dermis and throughout the subcutaneous tissues. These appeared to arise from or be closely associated with the small arterioles and capillaries. Some of the cells observed in the proliferating masses had large round to oval nuclei, finely granular to reticular chromatin, sharply defined nuclear membranes, and indistinct neutrophilic cytoplasm. These cells appear to be morphologically similar to the primitive mesenchymal cells described by Sevoian and Chamberlain.\(^5\) These masses usually contained a few plasma cells, heterophils and histiocytes. In some advanced lesions, many cells were hyperchromatic, pyknotic, and appeared to have coalesced to form dark lines and masses of cellular debris. In some instances the lesions were limited in size to just a few cells. In others, the lesions were so heavily infiltrated with inflammatory cells that they were difficult to classify with certainty. Cell changes induced by autolysis or excessive scald-water temperatures often rendered the tissues unfit for diagnosis.

It was not uncommon to observe that the surface epithelium was absent over the more massive underlying lesions.

**Nerve**

The lesion most commonly observed in the sciatic nerve was a focal to diffuse proliferation of mononuclear round cells between the fibers of the nerve bundle. The cellular aggregates were intimately associated with the small arterioles within the nerve bundle. Single to multiple cells often were interposed between individual nerve fibers in a longitudinal manner and gave the impression of a string of beads. In some lesions the proliferation was so extensive as to practically mask the architecture of the nerve. Nerve bundles in the skin were occasionally observed to be diffusely infiltrated with small hyperchromatic round cells.

**Brain**

Aggregates of round cells were observed surrounding the small arterioles and capillaries located in the white matter of the medulla, cerebrum and cerebellum.

**Heart**

Minor to massive foci of proliferating round cells were found in the epicardium, myocardium, and endocardium. These cells were frequently observed to be diffusely scattered between muscle fibers.
Liver

Round cell nodules, morphologically indistinguishable from normal "bursa dependent follicles" were observed in most liver sections examined. Massive foci of proliferating round cells were sometimes seen and were sufficiently extensive to obliterate the normal architecture. In a few instances the cell type present closely resembled the immature forms described in the skin lesions.

Kidney

Proliferating round cells were commonly observed in masses or diffusely scattered in the interstitial tissues.

Spleen

The lesions observed were primarily those of increased reticuloendothelial cells and cellular proliferation surrounding the arterioles.

Adrenal

Proliferating round cells were frequently scattered throughout the gland and in the ganglia surrounding the gland.

Gonad

Diffuse and nodular proliferation of round cells was observed in the interstitial tissues of testes and ovaries. In the more extensively involved organs there was an almost complete replacement of normal interstitial tissue. The proliferating cells did not invade the seminferous tubules of the testes or the ovarian follicles.

Discussion

The laboratory diagnostician frequently receives a small specimen of skin or a visceral organ from a "skin leucosis"-suspect chicken from which he is expected to render a diagnosis. In a busy schedule the temptation is present to process the specimen in a routine manner and to submit a report: a procedure which is usually a waste of time and effort.

Current evidence suggests that skin lesions are closely associated with visceral leucosis and that avian lymphomatosis is a systemic disease rather than one confined to specific anatomical structures. If these observations are true then it is unrealistic to base a differential diagnosis of acute avian leucosis solely on the presence of gross and microscopic lesions in the skin.

There can be wide variation in the size and numbers of affected feather follicles, as well as variations in the predominant cell types observed in microscopic sections of the skin. Also, tissue changes due to autolysis or thermal damage can be extensive. Folliculitis lesions may be present which grossly and microscopically resemble the skin lesions of leucosis and, therefore, require careful differentiation. To justify a diagnosis of acute avian leucosis in birds exhibiting skin lesions the
authors concluded that the skin lesions must be evaluated in relationship to co-existing nerve and visceral lesions. Specimens for gross and microscopic examination should include skin lesions, peripheral nerves and ganglia, brain, cardiac and skeletal muscle (if gross lesions are observed), gonads and other visceral organs.

Benton, et al.\textsuperscript{1} reported that approximately 74 percent of carcasses with skin leucosis also exhibited gross lesions of leucosis in the viscera while in this laboratory it was observed that only 16.5 percent of carcasses with skin lesions showed concurrent gross visceral lesions. This disparity observed in these two studies is not readily understood, but may be related to a variation in leucosis virus strain susceptibility among different genetic strains of chickens\textsuperscript{5,6} or, it may be due to possible differences in criteria used as a basis for classification under "skin leucosis." Regardless of the cause of the observed variation in incidence of concurrent skin and visceral lesions, it is well to keep in mind that the absence of gross visceral lesions in carcasses exhibiting skin lesions does not rule out the existence of microscopic visceral lesions in the same carcass. In the experience of the authors the converse is also true.

This study did not support the contention that skin lesions are more difficult to distinguish after the carcasses have been chilled. However, the numbers of birds tested for this phenomenon may have been too few for a valid conclusion.

The common occurrence of affected feather follicles irregularly located between the feather tracts was found to be very helpful in making a rapid gross evaluation of skin lesions. Gross and microscopic examination of these lesions did not reveal characteristic changes differing from those found in affected follicles located along the feather tracts.

Dougherty and Seibold\textsuperscript{3} reported that hard-scald temperatures (150°F) "fix" the stratum corneum and produce dermal changes that render identification of cell types quite difficult. This could explain the difficulty encountered in the gross detection of skin lesions underlying the yellow "bloom."

The greater incidence of skin and visceral lesions among females (Table II) supports the observation of Benton, et al.\textsuperscript{1} and was shown to be a highly significant difference by the Chi-square test.

SUMMARY

Data are presented on gross observations and microscopic lesions of acute avian leucosis in a random sample of 13,058 broiler carcasses condemned by Federal inspectors.

The observed incidence of concurrent skin and visceral lesions of leucosis supports the findings of other workers and justifies the contention that a differential diagnosis of acute avian leucosis must be approached on a systemic basis. Some diagnostic guidelines and difficulties are discussed.
REFERENCES

WORK ON MYCOTOXICOSES AT THE UNIVERSITY OF MINNESOTA

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and C. J. Mirocha, Ph.D.***

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Mycotoxicoses probably always have been important in Veterinary Medicine, but only since about 1962 have they been recognized as a potentially serious problem in animal health in the United States. Forgacs referred to them as "the neglected diseases," which certainly was true at the time he wrote, just a few years ago, but now mycotoxicoses are receiving increased attention. The first report on poisoning of poultry by aflatoxin was in 1962; within the next three years approximately 500 research papers dealing with aflatoxins were published.

Our work with mycotoxicoses was begun in 1963. My experience as a practicing veterinarian, and later in the Diagnostic Laboratories of the College of Veterinary Medicine of the University of Minnesota, had convinced me that mycotoxicoses might be involved in a number of common diseases of unknown etiology, diseases hitherto diagnosed as idiopathic.

Our work began with the estrogenic syndrome in swine. As you know, this is a widespread, common, and economically important disease, and at the time our work began the immediate cause of the syndrome was not known, although the syndrome was reported to be associated with the consumption of moldy corn. One outbreak of this disease occurred in an experimental herd of swine maintained by a large producer of commercial feed, and fed with pelleted feed produced by this firm. Other outbreaks occurred in swine herds on farms in Minnesota in the fall of 1963 and early winter of 1964. From some of these farms we collected samples of corn with which the affected swine were being fed. From this corn we isolated a number of fungi, including Fusarium. All of these fungi were grown in moist autoclaved corn for a time, then dried, ground, and fed to experimental animals.

Virgin female white rats 21 days old, when fed for seven days with corn inoculated with some isolates of Fusarium, developed uteri as much as 10 times as heavy as the uteri of control rats fed sound corn. Gilts six weeks old fed with this corn overgrown with the same strain of Fusarium, within seven days developed enlarged vulvas, mammae, and nipples, and one gilt, kept on this feed for 18 days, developed a prolapsed vagina. The uteri of these gilts were greatly enlarged, and the ovaries were atrophied. The gilts which had developed enlarged vulvas after having been kept on

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this feed for 18 days, were then put on a normal swine ration. They soon became normal in appearance, later cycled normally, and conceived when bred. If poisoning from this Fusarium has not progressed too far, affected swine may recover if put on sound feed.

Over the past three years we have tested in rats more than 100 isolates of Fusarium from corn collected on farms where the estrogenic syndrome was observed in swine, and over 50 of these isolates, when fed to rats, have resulted in greatly enlarged uteri within seven to ten days. There is no question in our minds but that the fungus Fusarium is a major cause of the estrogenic syndrome in swine. Doctor Mirocha has isolated, purified, and identified the estrogenic compound produced by Fusarium, and now has a sufficient quantity of it for tests with other animals.

Abortions in swine usually have been assumed to be due to leptospirosis, for the diagnosis of which a serological test is available. Our records do not support this assumption. In the past year, in our Diagnostic Laboratories, 2,789 serum samples were tested from herds of swine in which a high percentage had histories of abortions or infertility problems, or both. Only 76 or 2.8 percent, of these swine had antibody titers, and only 35 attained diagnostic titers of 1:1000 or higher. One hundred percent of the swine feti examined were bacteriologically sterile. Consequently, the routine diagnosis was reported as due to idiopathic abortions. It is reasonable to assume that some of these abortions may have been caused by the estrogenic compound produced by Fusarium. In one of our tests, four purebred Yorkshire gilts, all immunized against hog cholera, erysipelas, and leptospirosis, and which at the start and finish of the test were negative for brucellosis and leptospirosis, were fed as follows: the control gilt received normal sow ration; the others received feed containing respectively 25, 50 and 100 percent corn invaded by an isolate of Fusarium known to produce an estrogenic response in rats. The gilt receiving a ration containing 50 percent of corn invaded by Fusarium developed an enlarged vulva after four days, and aborted after 21 days. The control gilt weaned a litter of ten pigs, and the three gilts fed different amounts of corn invaded by Fusarium weaned a total of 11, or an average of 3.7 per gilt.

Fusarium is not just Fusarium. We have isolates of this fungus that are strongly estrogenic when fed to rats or swine. We have other isolates of Fusarium that, when grown for a time on autoclaved moist corn and fed to rats, result in death within a few days, and within that time they produce a 10- to 11-fold increase in weight of uteri of the rats. We have still other isolates of Fusarium that result in death of rats within a few days when fed to them, but which produce no detectable estrogenic response. According to other workers, some species of Fusarium when growing in grain make the grain unattractive to swine. We have not seen, from our feeding experiments any occasion where swine refused to eat moldy feed, even when 100 percent of the kernels were invaded with the fungi.

We have tested more than 1000 isolates of fungi for toxicity of rats. Most of these fungi have come from feeds suspected of harboring toxic fungi, feed from farms on which illness or death had occurred in swine,
cattle, or poultry, and which could not be attributed to any pathogenic agent or known chemical poison. Of these more than 1000 isolates, over 150, when grown in autoclaved moist corn and fed to rats, have been lethal within two to 14 days. Of 50 isolates of Penicillium from corn and from processed turkey feed, 40 have caused death of rats in four to six days. Twenty of 21 isolates of Alternaria, 25 of 26 isolates of Cladosporium, a number of isolates of Aspergillus flavus and A. ochraceus, six of six isolates of A. niger, and 10 of 12 isolates of Scopulariosis, and 25 of 53 isolates of Chaetomium globosum have caused death within four to seven days of rats to which they were fed. Symptoms have included haemoglobinuria, frank hemorrhaging into the lumen of the intestine, disturbances of the central nervous system, and greatly prolonged prothrombin time.

Production of toxins by these fungi is influenced by the strain or isolate of the fungus of any given species or genus; by the material in which the fungus is growing; by the combination of other microflora present; by the moisture content and temperature of the substrate; by the length of time the fungus grows; and by all possible combinations of these factors. We have more than 100 isolates of fungi that in our tests have produced metabolites lethal to rats. Some of these fungi are extremely common and prevalent in feeds, and a few of them are common in some kinds of human food. Obviously we have not been able to explore all of these fungi in depth.

Advances in this work will come only through more research. This research is complex and expensive, and progress inevitably is slow. But only if research of this sort is established and continued on a longtime basis can we in Veterinary Medicine fulfill our obligations to the animal industry of this and of other countries.

CHEMICAL DETERMINATION OF SOME MYCOTOXINS

The estrogenic factor produced by Fusarium and herein referred to as F-2 is a resorcylic acid lactone derivative with an emperical formula of C_{18}H_{22}O_{5} and believed to have the chemical structure as shown in Figure 1. The compound exhibits strong absorption maxima at wavelengths of 236, 274, and 314 mu (Figure 2). It emits a strong blue-green fluorescence between 440 and 460 mu when excited with radiation of around 310 mu making it easily adaptable to detection when separated by thin layer chromatography.

The estrogenic factor is soluble in n-butanol, ethanol, acetonitrile, chloroform and methylene chloride. It can easily be extracted from biological material with methylene chloride and then identified and quantitated by thin-layer or gas-liquid chromatography. Detailed methods of analysis have been worked out and are available.

The toxin produced by Chaetomium globosum has been isolated but has not been characterized nor the methods of its analysis as well defined as with the estrogenic factor. It can be extracted from corn colonized by Chaetomium with methylene chloride, chloroform and acetone. It is not readily soluble in petroleum ether (B.P. 60-80C), methyl
Figure 1. Chemical structure of the estrogenic factor (F-2) as determined by Andrews, F. N., U. S. Patent 3,196,019.

Figure 2. Absorption maxima of the estrogenic factor (F-2) in the ultraviolet region when measured in ethanol.

cellosolve or water. Preparations of this toxin when purified by column chromatography using silica gel as an adsorbent and injected into white rats intra-peritoneally result in death within 24 hours.

Work concerning the chemical identity of the other toxins herein described have not progressed far enough to report as yet.
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