PROCEEDINGS

SIXTY-NINTH

ANNUAL MEETING

UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

JACK TAR HOTEL
Lansing, Michigan
October 25, 26, 27, 28, 29, 1965
the E.I.A. virus. Research efforts must be expanded in the hope that an immunizing procedure can be developed.

During the last year the disease has appeared in race horses. On recommendation of the Department, the New York State Racing and Harness Racing Commissions have directed that special precautions be taken at all tracks in the state.

All reports of suspected cases are investigated and horse inoculation tests are conducted if indicated.

Recognizing that a solution of this disease problem can come only from expanded research, New York State has provided funds for this purpose. The work is now getting under way at Cornell University. Initial efforts will be directed toward the development of an accurate laboratory test for the detection of infected horses. Research efforts and control efforts should have the support of all horsemen and horse associations.

Q - What is the treatment for E.I.A.?
A - There is no known cure or useful treatment.

Q - What preventive and control measures can be taken?
A - Control flies, mosquitoes and other biting insects by screening, insecticides and good sanitary practices around the stable. Provide separate equipment for each horse such as bridles, girths, curry combs, brushes and watering pails. Isolate and carefully observe any purchased additions for at least 10 days before stabling with other horses. Sick horses should be examined by a veterinarian without delay. Don't use instruments that penetrate the skin unless boiled for at least 15 minutes.

Q - What is the New York State Department of Agriculture and Markets doing about E.I.A.?
A - Regulations have been put into effect requiring that all horses coming into New York be examined and their temperatures recorded by a licensed accredited veterinarian within 10 days prior to entry into the state.

Nelson A. Rockefeller, Governor
Don J. Wickham, Commissioner
New York State
Department of Agriculture and Markets
State Campus
Albany, New York 12226
Q - What is Equine Infectious Anemia?

A - Equine Infectious Anemia, commonly spoken of as "Swamp Fever", is a contagious disease of equines characterized by anemia, loss of condition, weakness, and sometimes death.

Q - What causes Equine Infectious Anemia (E.I.A.)?

A - This disease is caused by a virus with many unusual characteristics. Ordinary disinfecting methods will not kill it. It will even withstand boiling temperatures of less than 15 minutes duration.

Q - How does a horse become infected with this disease?

A - Infected and apparently recovered horses are carriers of the virus and transmission of minute particles of blood from these horses to susceptible horses by biting insects, contaminated instruments that puncture the skin such as hypodermic needles and tattoos can transmit the virus. Brushes, curry combs, bridles, etc. could also become contaminated and spread the disease. Secretions and excretions may also contain the virus.

Q - How long will a horse carry the virus?

A - Once infected, horses generally remain carriers of the virus for the rest of their lives. Reports list one horse as a carrier for 18 years.

Q - What animals does this virus infect?

A - Horses, ponies, mules and donkeys are the only known natural hosts of E.I.A.

Q - Where has this disease been found?

A - E.I.A. has been reported in all parts of the world, 43 of the 48 mainland states and most of the Canadian provinces. It is being reported more frequently now and may be on the increase. Recent increases in the number and value of horses has focused attention on all equine disease problems.

Q - What are the symptoms of E.I.A.?

A - Symptoms vary, but some of the following are usually seen: high fever (103° - 108°) that goes up and down; swellings of the lower portions of the body and on the legs; weakness, especially in the hind quarters; anemia; jaundice; loss of condition - although horse continues to eat. Death may occur within 2-4 weeks or the animal may seem to recover. Chronically affected horses appear unthrift and lack stamina. Relapses may occur in carrier horses when stressed by work, training or other diseases.

Q - How can E.I.A. be accurately diagnosed?

A - Call your veterinarian. The diagnosis of E.I.A. presents many difficulties. Continuous observations along with regular morning and night recording of body temperatures and regular blood studies are helpful. Additional research is needed to develop an accurate, practical test.

The only accepted and recognized test at present involves the inoculation of suspect blood into a test horse. The test horse must then be observed for a period of 60 days during which time temperatures are taken morning and night and routine blood studies made. Preinjection preparations of the test horse may consume an additional month.

Q - Is there a vaccine for E.I.A.?

A - No. Immunity in the true sense, as produced by most virus infections, is not produced by
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UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

JACK TAR HOTEL
Lansing, Michigan
October 25, 26, 27, 28, 29, 1965
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Sanitary Association

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1. Sept. 27-28, 1897**
2. Oct. 11-12, 1898
3. Oct. 11-12, 1899***
4. Oct. 2-3, 1900
5. Oct. 8-9, 1901
6. Sept. 23-24, 1902
7. Sept. 22-23, 1903
8. Aug. 23-24, 1904
9. Aug. 15-16, 1905
10. Aug. 15-16, 1906
11. Sept. 16-17, 1907
13. Dec. 5-6-7, 1910
14. Dec. 3-4-5, 1912
15. Dec. 2-3-4, 1913
16. Dec. 2-3-4, 1914
17. Dec. 2-3-4, 1915
18. Dec. 2-3-4, 1916
19. Dec. 2-3-4, 1917
20. Dec. 2-3-4, 1918
21. Dec. 2-3-4, 1919
22. Nov. 28-29-30, 1920
23. Dec. 6-7-8, 1921
24. Dec. 3-4-5, 1922
25. Dec. 3-4-5, 1923
26. Dec. 2-3-4, 1924
27. Dec. 2-3-4, 1925
28. Dec. 2-3-4, 1926
29. Nov. 30-Dec. 1-2, 1927
30. Nov. 5-6-7, 1928
31. Nov. 4-5-6, 1929
32. Dec. 5-6-7, 1929

Place of Meeting
1. Fort Worth, Texas
2. Omaha, Nebraska
3. Chicago, Illinois
4. Louisville, Kentucky
5. Buffalo, New York
6. Wichita, Kansas
7. Denver, Colorado
8. St. Louis, Missouri
10. Richmond, Virginia
11. Washington, D.C.
12. Chicago, Illinois
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President
1. Mr. C. P. Johnson, Springfield, Ill.
2. Mr. C. P. Johnson, Springfield, Ill.
3. Mr. C. P. Johnson, Springfield, Ill.
4. Mr. C. P. Johnson, Springfield, Ill.
5. Dr. E. P. Niles, Virginia
6. Mr. W. H. Dunn, Tennessee
8. Mr. M. P. Smith, Montana, Mo.
9. Mr. M. P. Smith, Monticello, Ill.
10. Mr. M. P. Smith, Springfield, III.
11. Mr. M. P. Smith, Monticello, Ill.
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33. Mr. M. P. Smith, Springfield, Ill.

Secretary
1. Mr. D. O. Lively, Fort Worth, Texas
2. Mr. Taylor Bridges, Kansas
3. Mr. T. I. Fishman, Louisville, Ky.
4. Dr. F. T. Olsen, Monticello, Illinois
5. Mr. W. P. Smith, Monticello, Illinois
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<tr>
<th>Year</th>
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<tr>
<td>1951</td>
<td>Oct. 12-13-14, 1949</td>
<td>Columbus, Ohio</td>
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<tr>
<td>1955</td>
<td>Sept. 23-24-25, 1953</td>
<td>Atlantic City, N.J.</td>
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<td>1956</td>
<td>Nov. 10-11-12, 1954</td>
<td>Omaha, Neb.</td>
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<td>Nov. 16-17-18, 1955</td>
<td>New Orleans, La.</td>
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<td>1958</td>
<td>Nov. 28-29-30, 1956</td>
<td>Chicago, Ill.</td>
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<td>Nov. 13-14-15, 1957</td>
<td>St. Louis, Mo.</td>
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<td>1960</td>
<td>Nov. 4-5-6, 1958</td>
<td>Miami Beach, Fla.</td>
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<td>1964</td>
<td>Nov. 30- Nov. 1-2, 1962</td>
<td>Washington, D.C.</td>
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<td>1965</td>
<td>Oct. 15-16-17-18, 1963</td>
<td>Albuquerque, N.M.</td>
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*Dr. A. E. Wight, Washington, D. C.
*Dr. J. W. Connaway, Columbia, Mo.
*Dr. Peter Malcolm, Des Moines, Iowa
*Dr. E. T. Faulder, Albany, N.Y.
*Dr. T. E. Robinson, Providence, R.I.
*Dr. Edward Records, Reno, Nevada
*Dr. Walter Wisnicky, Madison, Wisc.
*Dr. R. W. Smith, Concord, N. H.
*Dr. D. E. Westmorland, Frankfort, Ky.
*Dr. J. L. Axby, Indianapolis, Ind.
*Dr. H. D. Port, Cheyenne, Wyoming
*Dr. E. A. Crossman, Boston, Mass.
*Dr. I. S. McAdory, Auburn, Alabama
Dr. W. H. Hendricks, Salt Lake City, Utah
Dr. J. M. Sutton, Atlanta, Ga.
Dr. C. U. Duckworth, Sacramento, Calif.
*Dr. William Moore, Raleigh, N. Carolina
*Mr. Will J. Miller, Topeka, Kansas
*Dr. Jean V. Knapp, Tallahassee, Fla.
*Dr. T. O. Brandenburg, Bismarck, N. D.
Dr. C. P. Bishop, Harrisburg, Pa.
*Mr. F. E. Mollin, Denver, Colo.
Dr. Ralph L. West, St. Paul, Minn.
*Dr. T. Childs, Ottawa, Canada
*Dr. T. C. Green, Charleston, W. Va.
Dr. H. F. Wilkins, Helena, Montana
Dr. A. L. Brueckner, Baltimore, Md.
Dr. G. H. Good, Cheyenne, Wyoming
Dr. John G. Milligan, Montgomery, Alabama
Mr. F. G. Buzzell, Augusta, Me.
Dr. J. R. Hay, Chicago, Ill.
Dr. A. P. Schneider, Boise, Idaho
Dr. W. L. Bendix, Richmond, Va.
Dr. T. J. Grennan, Jr., Providence, R.I.
Dr. L. A. Rosner, Jefferson City, Mo.
Dr. J. W. Safford, Helena, Mont.
*Dr. O. E. Dyson, Wichita, Kansas
*Dr. O. E. Dyson, Wichita, Kansas
*Dr. O. E. Dyson, Wichita, Kansas
*Dr. O. E. Dyson, Wichita, Kansas
*Dr. O. E. Dyson, Wichita, Kansas
*Dr. L. Enos Day, Chicago, Ill.
*Dr. L. Enos Day, Chicago, Ill.
*Dr. L. Enos Day, Chicago, Ill.
*Dr. L. Enos Day, Chicago, Ill.

****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 Sixty-ninth 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
THE HISTORY OF REGULATORY VETERINARY MEDICINE
IN THE UNITED STATES

R. A. Hendershott, Secretary-Treasurer

The written history of regulatory work in the United States as it pertains to diseases of domestic animals goes back more than one hundred and fifty years. As a matter of fact, as early as 1785 we find that the State Legislature of North Carolina passed a law requiring that no cattle should be moved within that State from April 1 to November 1 of each year.

In the year 1860, the Agricultural Division of the Patent Office reported the prevalence of hog cholera among our swine. This, we believe, was the first report of the National Government calling attention to the prevalence of an animal disease in this country. Just two years later, in 1862, by Act of Congress, the United States Department of Agriculture was established. By 1869 the losses from animal diseases were attracting widespread attention, but up to that time the National Government had given no assistance to the livestock raiser to combat these diseases. However, Mr. Horace Capron, then the United States Commissioner of Agriculture, stated: "The numerous epizootic and zymotic diseases by which our cattle are infected demand the intelligent consideration of the Government and the several States. The experiences of the past few years have demonstrated the necessity of such facilities and I therefore strongly recommend the establishment of a Division of Veterinary Surgery in connection with this department." In 1870 Commissioner Capron repeated his recommendation and finally on May 1, 1883, Dr. S. Salmon was called to Washington to establish a Veterinary Division of the Department of Agriculture. The Veterinary Division was established immediately with the view of providing facilities for the investigation of animal diseases.

Anthrax, black leg, contagious abortion, fowl cholera, hog cholera, pleuropneumonia, sheep scab, Texas Fever, tuberculosis, and other animal diseases had existed in this Country for years. There had also been an outbreak of foot and mouth disease. Heavy losses were reported each year from various sporadic diseases, and unfavorable conditions had arisen in connection with our export trade. All of which presented an urgent need for the establishment of a veterinary organization in the Department of Agriculture, provided with funds and authority to control the spread of communicable diseases, as well as to prevent the importation of foreign plagues, and most of all to enable us to secure free entrance of our animal products into the markets of the world.

The presence of pleuropneumonia in some of the Eastern States and the necessity of eradicating it in order to prevent its spread to the herds of the West, and to protect our export cattle trade, was one of the factors leading to an Act of Congress that established the Bureau of Animal Industry, now the Agricultural Research Service. Although contagious
pleuropneumonia was known to have existed in this country as early as 1843, the importance of eradicating it was not fully appreciated until 1879.

Texas Fever was another disease that entered into the early history of this country, and that with hog cholera (1833) coupled with pleuropneumonia no doubt were the deciding factors that led to the creation of an organized veterinary service. There appears to be no question that Texas Fever was introduced into this country through importation of cattle from Spanish colonies, but history tells us that our first accounts of Texas Fever did not come from these sections, but from localities where it had been carried by the movement of cattle from the South. The earliest published accounts that we find of the disease was in a lecture delivered by Dr. James Mease on November 3, 1814 before the Philadelphia Society for Promoting Agriculture. In 1814 the State of Virginia refused to allow cattle from certain sections of South Carolina to pass through the state. Although these laws were strengthened from time to time, during the next forty two years Texas Fever spread to the Blue Ridge Mountains and became planted even beyond this natural barrier. With the extension of settlements in these early years and the growth of the livestock industry and transportation facilities, the disease gradually spread over the entire South and to different sections of the North. Little was known of the disease in the Northern states prior to 1866. By 1867 it was the cause of much alarm throughout the country and especially along the avenues of transportation and at the centers of the cattle trade. Along the old cattle trails from Texas and other Southern states animals driven to Northern markets and feeding grounds, invariably left disease and death in their wake. By far the greatest losses seemed to have been occasioned by driving cattle through Missouri, Kansas, Arkansas and the Indian Territory for distribution as feeders in various Mid-western states. In 1861 the state of Missouri passed laws to regulate the movement of herds from the South. Other states soon followed the example of Missouri and the people of these states were aroused to great indignation because some Texas cattlemen persisted in driving their herds North. This resulted in armed parties turning and driving back the invading herds. Such vigorous enforcement of the law, however, had a beneficial effect in preventing further losses.

The disease ceased in all of these border states during the Civil War, but immediately after the close of the war, when Texans again sought an outlet through Kansas and Missouri for their accumulated livestock, it reappeared. In order to avoid the hostile opposition met in the adjacent states, Texas cattlemen were forced to ship their livestock up the Mississippi River by boat, unloading them at Cairo, Illinois. Many of the animals were reshipped by rail from this unloading point, spreading Texas Fever eastward from Illinois to Massachusetts.

The history of hog cholera and other contagious and infectious diseases which influenced the pattern of regulatory medicine in the United States followed about the same course. Therefore, it is evident that back in these early years, not only our National Government, but the several
states accepted their responsibility in the control and eradication of the contagious and infectious diseases that affected the livestock industry at that time. The best informed livestock owner and the veterinary authorities came to realize that a national direction of the activities for the extermination of the malady would overcome the worst and most discouraging features which would prevent the efforts of the individual states from being effective.

In an article published early in 1879, Dr. James Law of Cornell said: "It matters little whether a contagious and infectious disease is controlled by the State or the National Government."

In 1870 our livestock industry had grown to such an extent that it seemed extremely desirable to find an outlet in foreign markets. In 1875 the first shipment of this kind was made. From then on our export trade continued to grow. American meats were becoming more and more popular in foreign markets, and the trade was carried on without interruption until 1879, when it was alleged that English Veterinarians had found lesions of contagious pleuropneumonia in a cargo of cattle that had been shipped from Portland, Maine to Liverpool. Because of this, orders were issued that all American cattle arriving at English ports must be slaughtered within ten days on the docks where landed. Further restrictions were placed upon our exports, and as stated above, these restrictions together with the fact that contagious and infectious diseases were being spread from state to state, were the main factors that prompted the Congress to organize the Veterinary Professional Service, in order that these several diseases could be eradicated and controlled with speed and efficiency. However, the organization of the Veterinary Service was not established without a lot of opposition.

The growth of the livestock industry had been rapid and enterprising stockmen were continually importing purebred animals from abroad to improve their herds. The glowing prospects of an extensive export trade in animals as well as their products gave a boost to the livestock industry and brought the people in general to realize more fully the importance of the livestock phase of agriculture.

But, up to 1883, the livestock industry has been left to drift without much assistance from either State or National Governments. The cause of many of these destructive diseases were unknown, or at least they were in dispute, and the livestock owners were more or less defenseless, as veterinary science in these early years had not been able to provide effectual prophylactic or medicinal treatment. Our system of interstate and export animal transportation were being challenged on every side and denounced as a disgrace and an outrage and then, as now, there was a growing demand for the protection of the public health of our people in connection with the meat supplies and our dairy products. Our export cattle were being denied admission into Great Britain and our pork was being prohibited entrance into most of the markets of Continental Europe.

There was an urgent need of reliable official information concerning the nature and prevalence of animal diseases. These were the conditions that faced the Regulatory Officials and the veterinary profession during
the latter half of the last century. It soon became apparent to the livestock industry that there was a need of an efficient agency, both National and State, to carry into effect laws and regulations essential to the control and eradication of livestock diseases as well as to protect and develop the rapidly growing industry here at home and the export trade.

So it was that on May 29, 1884, by Act of Congress, the United States Bureau of Animal Industry was established. But history points out clearly that the bill creating the Bureau did not go through Congress without serious opposition; then, as now, there were lawmakers jealous for the right and powers of the states. There were also those in Congress who ridiculed the veterinarians and their knowledge of livestock diseases. Many saw in the proposed organization nothing but an army of job holders or a political machine. Even the presence of pleuropneumonia in the United States was doubted and questioned. In fact, the veterinary profession was at that time struggling for recognition in America. The profession, however, was not without friends and the bill was championed on the floor of the House of Representatives by the Hon. William H. Hatch of Missouri, then chairman of the Committee of Agriculture. History tells us that Mr. Hatch patiently explained the various features and provisions of the bill and skillfully met every objection.

In the course of his speech in defending the veterinary profession he referred to almost every veterinarian in prominence living on foreign soil. Coming to America, he stated, and we quote in part: "we find that in spite of the admitted fact that the most destructive diseases of people can never be thoroughly understood until more is known of the disease of animals; in spite of the annual loss of more than fifty million dollars from preventable diseases among animals in the United States, the veterinary profession has been held in the background, discouraged and derided by those who should have assisted and defended it. Even in the Congress of the United States, which is supposed to represent the intelligence and the most progressive sentiment of the country, such terms of reproach and scorn as horse doctor and scientific cranks are hurled at those who are trying to prevent these enormous losses from falling on the livestock industry and to assist in throwing light on the most destructive plagues of the human race. The American Veterinary profession, although few in numbers, begins to rank in ability and scientific attainments with any in the world. It is true the profession is still small but it is rapidly growing; it has fought its way against every obstacle and discouragement; its members have sought education and knowledge in every department of science and in every language where assistance is to be found; and the appreciation of its usefulness, beginning with the masses, has permeated all classes of our society, until praise and favor have taken the place of insolence everywhere, with the single exception of the House of Representatives of the United States."

Up to this point, we have dealt with a few of the conditions that led up to the organization of the veterinary profession for the sole purpose of controlling and eradicating contagious and infectious diseases amongst the livestock industry. It is evident that laws, rules and regulations governing
the conduct of the industry were put into effect for the sole purpose of assisting in combating livestock diseases as well as to meet certain demands of the export and import trade. We have discussed a few of the diseases that were prominent in making these decisions. One of them was Texas Fever, and in our review of early history, we find that this disease was primarily the cause of the organization of what is now one of the largest and most influential organizations dealing with rules, regulations and scientific approach to the disease problems of the livestock owner, and we refer to the Interstate Association of Livestock Sanitary Boards now known as the United States Livestock Sanitary Association.

In the latter years of the last century the North-Central States were obliged to prohibit the importation of cattle from the South during the warm months of the summer because of Texas Fever. These regulations deprived the Southern ranchmen and livestock owners from driving their cattle into the prairie states where there was abundance of feed and a few of the regulatory officials of these states were invited to hold their meeting in Fort Worth, Texas, on September 27, 28, 1897, to discuss ways and means whereby this condition could be rectified. This little group eventually organized itself under the name of "Interstate Association of Livestock Sanitary Boards," and held yearly meeting until 1909, when the name of the Association was changed to the United States Livestock Sanitary Association. One of the functions of this organization is to recommend uniform rules and regulations governing the movement of livestock and to recommend national policies, based upon scientific research, pertaining to the eradication of contagious and infectious diseases of our domestic animals and poultry.

Because of the activities of this organization, the livestock industry has received the benefit of a unified effort, with the result contagious pleuropneumonia, foot and mouth, dourine, glanders, vesicular exanthema has been eradicated and tuberculosis, brucellosis, hog cholera are fast approaching eradication. The philosophy of the United States Livestock Sanitary Association, which could be better called the American Animal Health Association, has always been to eradicate a disease as opposed to living with and paying a tax to it.

These and other great achievements could never have been realized without the cooperation of the livestock owner, our livestock organizations Veterinary Research Workers as well as the veterinary profession. Someone has said that livestock is the foundation of America's agricultural wealth and that veterinary medicine is its greatest safeguard.

With livestock losses due to diseases and parasites amounting to the fabulous figure of $2,688,000,000 annually, the Veterinary profession and the Regulatory officials, both State and Federal, face a real challenge. The American Veterinary Medical Association, the United States Livestock Sanitary Association, the private practitioner, and the Regulatory Officials of all stages, together with the livestock owner, cooperating, has met and I am confident will continue to meet the challenge of disease and conquer it.

The price of continued freedom from the returning source of the
ravishing diseases of our domestic animals which have been practically eradicated, as well as the pressing need to wage increasing warfare against other dangerous and economic livestock diseases, demands of us constant watchfulness research and service.

Undoubtedly, disease eradication accomplished to date has materially accounted for more efficient production of food and fiber of animal origin.

The development of "Specific Pathogen Free" animal populations holds great challenge and opportunity in the years ahead. Currently the achillies heel in animal disease control and eradication is our relatively weak defense against the introduction of foreign animal disease.

The opportunity for introduction of foreign plagues has been greatly increased by modern means of transportation and the opening of the Lakes to Seas Water route. Let us not overlook the possibility of introduction from foreign lands through the activity of government agencies employed in rendering aid in far off places due to the traffic back and forth.

We have come a considerable way since 1884, we have excellant re-search workers in and out of government, to provide the answers to ques-tions we must have the answers to in order to devise control and eradica-tion procedures which are rectified in the crucible of open committee meetings attended by those livestock owners that may be governed by our actions. We have been left a responsible heritage it is our duty to guard and improve upon it; in achieving this goal we are fortunate to have meeting with and assisting us The Conference of Laboratory Diagnosticians.
ADDRESS OF WELCOME
W. W. Armistead, Dean
College of Veterinary Medicine
Michigan State University

INTRODUCTION

Although justly famous as the automobile capital of the world and as one of the nation's great tourist spots, Michigan also is an important agricultural state. Her total cash agricultural receipts exceed $763 million per year and her total "agribusiness" is estimated at $2 billion per year. Michigan ranks as number one in the production of navy beans, pickles, tart cherries, and is at or near the top in the production of a dozen other crops.

Michigan also is an important livestock state, placing eighth in milk production among the 50 states. Milk is Michigan's number one farm crop and accounts for 28 percent of total cash farm receipts. The state also has thriving industries developed around beef cattle, poultry, swine, sheep, and horses.

EDUCATION IN AGRICULTURE AT THE COLLEGE LEVEL IS AN AMERICAN INVENTION

College-level agricultural education was invented in the woods of frontier Michigan 110 years ago, just five miles from this spot, in what is now East Lansing. In Michigan, which had only achieved statehood in 1837, the Michigan State Agricultural Society was founded in 1849. In this same year was held the first Michigan State Fair, where one E. H. Lothrop made an eloquent plea for a school where a young man could master the agricultural sciences.

There were in 1849 almost 200 colleges and universities in America, but none taught agriculture. Few taught more than the rudiments of chemistry or biology. These colleges and universities were modeled after their European forebears, with emphasis on Greek, Latin, and mathematics. Such education was intended primarily for clergymen, lawyers, and scholars.

At the request of the Michigan Agricultural Society, Bela Hubbard, a Detroit naturalist and farm owner, drafted a proposal for a revolutionary new kind of college whose objectives would be to:

1. Train the mind to observe, judge, and draw conclusions.
2. Teach agriculture and all the natural sciences.
3. Teach mathematics, bookkeeping, engineering, architecture, and landscape gardening.
4. Not neglect literature and fine arts "as tending to polish the mind and manners, refine and taste, and add greater lustre and dignity to life."
5. Have an experimental and model farm where students could discover "a practical application of the precepts taught."

Hubbard's concept was so logical, and so dignified, that it gained wide support and on February 12, 1855, Governor Bingham signed the bill establishing "The Agricultural College of the State of Michigan." The act specified that the following subjects be taught:

- Natural Philosophy
- Chemistry
- Botany
- Animal and Vegetable Anatomy
- and Physiology
- Geology
- Mineralogy
- Meteorology
- Entomology
- VETERINARY ART
- Mensuration (applied geometry)
- Leveling and Political Economy
- Bookkeeping
- Mechanic Arts

Interest in the new agricultural college idea spread and other states began to clamor for similar institutions. Finally, in 1862, President Lincoln signed the Morrill Act which gave federal land to each state for the support of colleges which would teach agriculture and the mechanic arts. These schools, of which M.A.C. was the pioneer, became known as the "land-grant" institutions. Michigan Agricultural College later became Michigan State College, then, on its 100th anniversary, it became Michigan State University.

**IMPORTANCE TO ANIMAL AGRICULTURE**

The development of the land-grant colleges was of immense importance to animal agriculture. It raised the scientific status of agriculture. It fostered agricultural research. Professional veterinary education and research in America "grew up" with scientific agriculture. Nearly all of today's veterinary schools are administered by land-grant institutions. It seems particularly appropriate, therefore, that this meeting of the United States Livestock Sanitary Association should be held here in central Michigan, where the immensely productive partnership of agricultural and veterinary sciences began more than 110 years ago.

**MSU, THE PIONEER LAND-GRANT COLLEGE, CONTINUES TO GROW—AND PIONEER**

Greater Lansing's largest industry is the Oldsmobile Division of General Motors. But its second largest industry is Michigan State University. MSU is eighth in the country in enrollment—over 35,500 this fall. The University's annual budget exceeds $100 million and the East Lansing campus covers more than 5,000 contiguous acres. It has 12 colleges, 70 departments, 200 different degree programs. MSU has educational development programs in a dozen foreign countries. It is a dynamic university with a worldwide reputation for successful educational innovation. But it is a university where agriculture still is a growing, rather than a
dwindling, concern. One evidence of this is the remarkable recent growth of buildings for agriculture. Under construction or completed during the past 18 months are the following major facilities:

1. $500,000 building for School of Packaging
2. $4,500,000 food science building
3. Plant radiation laboratory
4. Forestry building
5. $250,000 Beef Cattle Research Center
6. $4,700,000 Veterinary Teaching and Research Hospital

THE COLLEGE OF VETERINARY MEDICINE

These are particularly exciting days for the College of Veterinary Medicine at Michigan State University. We have a unique new curriculum, and a rapidly expanding faculty. We are embarking upon a new partnership with human medicine under a unique administrative organization. The College of Veterinary Medicine and the College of Human Medicine are sharing academic departments, facilities, and faculties, and in many cases students will take common courses in pre-clinical subjects. The long-range implications of this intimate integration of human and animal medicine are exciting to contemplate.

The College of Veterinary Medicine also has a mushrooming research program, including major new research efforts in:

- Tuberculosis
- Leukemia
- Gnotobiosis
- Nutritional pathology
- Primate anatomy
- Reproductive physiology

FINALLY

Let me extend a cordial welcome to each of you from the Greater Lansing Community, from Michigan State University, and most especially from the College of Veterinary Medicine.
Thank you, Dr. Safford. On behalf of the Michigan Veterinary Medical Association, I want to add my welcome to that of Dr. W. W. Armstrong, our Dean of the College of Veterinary Medicine. It is not often a dairy practitioner has this many bosses to talk to at one time. The talk that I am going to give now, probably is a lot different than that which I would have given in the field a number of years ago in the middle of the Bang's clean-up, when I had an angus herd to test and the only restraint was an apple tree in the middle of a 30-acre field surrounded by a one-wire electric fence.

I asked Doctor Quinn what in the world he wanted me to talk to this group about. He said that it didn't make any difference what I talked about because the people here were going to use that period for a rest period or a coffee break anyway.

Veterinary medicine really has many facets, many groups—there are the cattle practitioners, the equine practitioners, the regulatory people, bacteriologists, parasitologists—in fact there are about five ancillary groups in the American Veterinary Medical Association. To provide better service for clients and to provide a more rapid and worthwhile transfer of information, these groups are necessary; but there are many areas that we can handle much better together. You and I, as regulatory people and as livestock practitioners, certainly have this one thing in common and that is we owe our existence to the livestock and agricultural industry.

The greatness of any country, I think primarily is based on the efficiency of its agriculture. They tell me that in some of our underdeveloped countries at least 80 percent of the people are engaged in farming just to provide food for the population. I had a chance a few years ago with one of these people-to-people agricultural missions to go behind the Iron Curtain; and in Russia perhaps 25 percent of the people are engaged in agriculture. It was in September of 1962 and we had a chance to watch, down in one of the farms, a corn harvesting operation, and there were 75 people supporting one two-row corn picker. Just imagine that—75 people. It takes four or five here in our country. We had a chance to go up in Poland—it was raining, they took us out to a State Farm, muddy, they took us into a dairying set-up—we walked into one barn and there were probably 35 or 40 real nice-looking Holstein cows there. We asked them what those cattle were—why they were in the barn? And they said those were our tuberculous reactors. Aren't you going to sell them? "Well, yes, but we're going to milk them out first." They told us later that approximately a third of the cattle in Poland were infected with tuberculosis, and to
unload all those cattle and clean up all at once would ruin the industry—would ruin their economy.

Here in America, we're down to seven percent of our population providing food; this is rapidly within the next few years going to five, and they estimate by the turn of the century that only three percent of our people will be engaged in agriculture. This means that we have somewhere between 93 percent, and maybe 97 percent, of our population released for other endeavors—for industry, for research, for building, for elevating our standard of living. But the irony of this whole thing is that as agriculture becomes more efficient, they have less to say about their own destiny. A year ago when the Supreme Court came out with this one-man, one-vote edict, they ordered the States to reapportion. Michigan was one of the first ones to do this, and the result as far as agriculture was concerned, and of course as far as the veterinary profession is concerned, because we depend so much on agriculture for support, was quite disastrous. We had a 50 percent turnover in the Legislature—now, why am I bringing this up—because you either see it happening in your state, or you will. I read in the paper last night where the Kansas Supreme Court ordered their state to reapportion by next Spring.

As the profession in Michigan started looking around last Spring for support to defeat a Bill that was proposed (it was a bill to put a sales tax on service, which would have meant that every large animal practitioner would have to go down the road perhaps with a cash register in his front seat to make change. When I started looking around for support, the old agricultural people that we had always counted on were gone. The new legislature was consumer-oriented, democratic, labor-dominated, and there just wasn't support there to get. It so happened that there were enough people against it so that the Bill was defeated anyway—but it certainly shook us up, because we had actually nowhere to turn. So, as a profession we pretty near had to start again at the bottom building up our contacts. We tried to strengthen our relationship with agriculture and agriculture—let's not forget—is the second largest industry yet in Michigan—and they carry a lot of weight when they aren't fighting among themselves, but I have seen odd things. I have seen the Grange and Farm Bureau setting down at the same table, and as powerful as the Farm Bureau is in Michigan, I don't think they could get a Bill across alone themselves—simply nothing will ever get across that the Farm Bureau doesn't want; but when agriculture goes before the Legislature with a united front they can pretty near get their Bills passed.

We have associated ourselves with the other professions—we have an organization called the Michigan Association of Professions—medicine, dentistry, law, veterinary medicine, pharmacy, architecture and engineering—any any time a Bill comes up that affects two or more of the professions, they will all go to bat for us. But we have done our best work, and probably the most important work through local contacts, personal contacts. All their locals this month have invited their legislators in and talked to them—got acquainted with them. We're going to have to start from the grass roots level. This isn't an attempt in any means to take
over the Government, but it certainly is an attempt to get a line of communications from our people through to our representatives. I think if we're going to have a voice in our own destiny, as individuals, as a profession, and probably with the industries that we serve, our profession is going to have to take an interest in politics; not only take an interest, but to be informed and to be active. I think professional isolationism as we have known it, and as we have been able to set back and allow the industry to take care of us is dead. We're going to have to do it ourselves because there isn't anyone else around to do it.

This is a great industry that we're privileged to serve; you can't realize exactly how great it is until you have had a chance to see the industry in some of the other countries. If you don't get anything else out of this talk, I would like to have you when you go home, and you and your wife perhaps go to a Supermarket to do your shopping, just look around in this Supermarket at the abundance, the quality, and the variety of food; and then you look around and be thankful—and while you're there, maybe just take an instant and look up and rejoice.
RESPONSE TO WORDS OF WELCOME BY DR. ARMISTEAD

W. M. Thompson
Phoenix, Arizona

Doctors Johnson and Armistead, I speak for this Association when I say that we appreciate very much those very fine words of welcome to the great State of Michigan and to this beautiful Capitol City of Lansing.

And now, may I say just a little "pun" before I go on with what I intended to say when I got up here? The nickname, "Water Winter Wonderland" sticks in my mind, and I cannot get rid of the thought that if we in Arizona had this much water, either winter or summer, that state would really be heaven; and now let us hasten to remind you that this Association will meet out there in sixty seven.

It seems to be customary in this organization to have a westerner respond to the welcome address when we meet in the Eastern part of the country (although I am aware that this is also called the Middle West) and to reverse this situation and have an Easterner or Southerner respond to a welcome address when the meeting is held in the Western United States.

I remember two years ago when this Association met at the Western Skies Hotel at Albuquerque, we were given a hearty welcome by our long time friend and colleague, Dr. F. L. Schneider, who was as he put it "pinch hitting" for the Hon. Clinton P. Anderson, United States Senator from New Mexico, who was unable to fill the prearranged appointment to welcome the United States Livestock Sanitary Association to the State of New Mexico.

Dr. Schneider, as is his nature, gave us a really rousing welcome with much wit and story telling incorporated into his address.

After Doctor Schneider's warm words of welcome, Doctor Campbell of Florida responded. Doctor Campbell referred to the fact that he had thought his own state of Florida was vast until he observed the size of the State of New Mexico and looked out over thousands of square miles of the state from the summit of the very high Sandia mountain peak, and then he had decided that Florida was only half-vast by comparison.

Being from Arizona, one of the most vastly states in the Union, I perhaps could make a similar comparison regarding Michigan, but after looking at this state at close range for this, my second time, I would be sticking my neck out entirely too far to even try to do this, for I say to you that Michigan really has "IT." I hope that the people of Michigan who are in the audience will not think me too presumptious in attempting to tell you about your own state. Michigan is vast in many, many ways.

We in the United States rely on the people of Michigan for many of the good things of life. On my first trip to Lansing, not quite a year ago, I drove out of the General Motors Factory, just a few blocks from this hotel where one of the finest automobiles which can be purchased anywhere
in the world is built. By the way, I drove the car back to this meeting in order that I can see some more of this great country.

In addition to manufactured goods, Michigan is great because of its livestock industry; its colleges, including, Dr. Armistead, its College of Veterinary Medicine here at Michigan State University; and for its namesake "Water Winter Wonderland."

To go back for a moment to the livestock industry of Michigan, as has been stated in these addresses on other occasions, we know that any particular state has a progressive and therefore a great livestock industry when it sends to this assembly year after year a representative such as your able State Veterinarian, Doctor Quinn, who is also serving at the present time as one of the hard working officers of this Association.

I could say no more regarding the great livestock industry of your state than Doctor Quinn has already implied even if he didn't say it in so many words; and that is, that few cattle which are shipped out of Michigan ever reach their intended destination for the simple reason that they are of such superior quality that some one in another location invariably stops to look at them while enroute and buys them on the spot before they have a chance to reach the place of intended destination.

We can truthfully say that quality livestock is a trade mark of Michigan. Those of us who have driven here have seen first class livestock all over this state. Recent information states that the Livestock Improvement Association of Michigan is an organization of organizations, and is composed of at least ten livewire organizations which actively represent every facet of the livestock industry. A major aim of the Livestock Improvement Association of Michigan is to increase production of red meat in the state in order to more nearly produce the amount consumed by the large population of Michigan.

The United States Livestock Sanitary Association is vitally interested in this livestock industry phase of your existence here in Michigan, just as we are interested in a healthy livestock industry the World over. The history of this organization is a long and honorable one; and it is due in a big measure to the efforts of this organization that the healthy livestock industry of this country is the envy of the entire world. Certainly, also, Michigan State University has contributed its share in eradicating livestock diseases from America. Research here on bovine tuberculosis alone has put us where we are today in this all out effort to eradicate tuberculosis.

As this is the first time for a convention of this Association in Michigan, we as members can all say, that just as Doctor Armistead has welcomed us to this state, the officers and members of the United States Livestock Sanitary Association welcome this opportunity and your gracious invitation to visit this "Water Winter Wonderland." I can tell you that as a desert dweller I am very happy to see so much water.

We are glad we are here in Lansing with you today, Doctor Armistead. We thank you sincerely from the bottom of our hearts for inviting us. I know each of us can sense a very successful meeting here this week and we are looking forward to coming back on other occasions in the fruitful years ahead for this Association. Thank you.
PRESIDENT'S ADDRESS

J. W. Safford

Helena, Montana

Distinguished guests, members and friends at this, the 69th Annual Meeting of the United States Livestock Sanitary Association.

I would like to depart from the customary President's Address. In reviewing presidential addresses, it was found that many extolled the history of disease-control successes. This is good, and since the subject is now well documented in the Proceedings of this organization I will omit a repetition. I will also omit a review of current livestock disease-control activities, their aims and goals, because these will be very well presented by the reports and recommendations of the hard-working committees of this Association.

We can report—with pride—that the livestock health in these United States is good. As a direct result of this livestock health status, we can—again with pride—report that the food supply of animal origin is abundant, wholesome, safe and nutritious. We know this did not "just happen." This most important, most favorable situation could not have been possible without the control of livestock diseases. The control of livestock diseases would not have been possible without the support of the people in each of the 50 states of this great and wonderful country of ours. The challenge to those given the responsibility to keep it this way is great, is vital to the health of the nation, and is increasingly complex. The members of this Association must rededicate themselves to meet this challenge.

This—the 69th Annual Meeting—terminates a period of four successive years for me of being given the honor and opportunity to serve as an elected officer of the United States Livestock Sanitary Association. I believe it is expected of me to share with you ideas I may have gained from this experience on how we can perform better in the future. I shall take advantage of this expectation by making two recommendations—recommendations which, in my humble opinion, deserve the study and action of this organization.

The first recommendation I would make is that the present custom of having a "lame-duck" president make this address be discontinued. I believe this Association should know what the president—just elected—hopes to accomplish during his term of office—not report to you two days before termination of his office. I would therefore recommend that the president-elect give the presidential address at the Annual Meeting at which he is elevated to president. He would then be able to formally declare the goals and objectives he and the Association hope to accomplish during his term of office.

The second recommendation I will make requires most serious and immediate consideration of this organization. The United States Livestock
Sanitary Association is the logical organization to evaluate this recommendation. It is the only organization primarily made up of official members of livestock disease-control officials representing each of the 50 states. It is an organization of livestock disease-control officials who have a dedicated responsibility to each of their respective states and collectively a dedicated responsibility to their United States.

There is no better qualified livestock disease-control official than today's veterinarian who knows intimately how to approach and how to most effectively control and eradicate livestock diseases in his own state in which he has made his home through his own choice. I am convinced that every such dedicated veterinarian knows that the more his services can contribute to his home and to his state the more his state can contribute to the greatness of the United States. The opportunity for this service must always be provided and encouraged, not depressed or supplanted by the activities of a far-removed, sometimes over-zealous centralized agency. The opportunity must always be provided, and encouraged, for such dedicated veterinarians and their states to contribute to the greatness of their country—not to expect their country to do the job for them.

History has repeatedly shown that a nation that cannot feed itself can no longer remain strong and independent. History has repeatedly shown that livestock diseases, uncontrolled, can and will destroy a nation's source of food. History has repeatedly shown that there is only one way to combat, control, or eradicate livestock diseases and that is by the application of sound veterinary medical principles by skilled veterinary medical scientists.

We have been and are now facing the grim reality that there are inadequate numbers of veterinarians available to safeguard this nation's source of food and to assure a safe food supply. The prospect for the next twenty years, unless immediately corrected, is that the situation will become more critical. It has been reliably estimated that the United States will need at least 47,250 veterinarians by 1980—more than twice the present number. Utilizing present educational facilities to a maximum would still leave a shortage of 7,000 to 8,000 by 1980. This nation cannot afford the risk of such a shortage.

There are two things this Association must do to help avoid this risk. The United States Livestock Sanitary Association must use its influence to expand present schools of veterinary medicine and create new schools of veterinary medicine. In attempting to improve the quantity of veterinarians, a warning—perhaps unnecessary—should be put out to NOT lower the high standards of veterinary medical education.

The second thing the United States Livestock Sanitary Association must do, as I see it, is to make certain that the veterinary medical man power available in this country is utilized to a maximum, that there is no wastage of this resource, no duplication of effort. In my opinion there EXISTS a waste of this resource and a duplication of effort. It should—and must—for the benefit of the people of our states and nation be corrected. This has been called to the attention of the United States Livestock...
Sanitary Association formally through presidential addresses and informally through discussions in the halls of every Annual Meeting I have attended.

Dr. T. O. Brandenberg in his President's Address to the 53rd Annual Meeting of the United States Livestock Sanitary Association in 1949 stated, "The wastage due to this double-headed system is appalling and takes its toll of our top veterinary man power as well as huge sums of money uselessly used up in maintaining two offices in every state with more-or-less duplicated records and work on all cooperating projects." Doctor Brandenberg requested a committee be appointed and a report the following year. No committee was appointed, no report exists.

President F. Mollin in 1951 requested this committee be appointed. Again no committee, no report.

Doctor Brueckner in his President's Address to the 60th Annual Meeting in 1956 refers to Doctor Brandenberg's recommendation to avoid state and federal duplication and makes this statement: "Strong state-supported organizations for diagnosis and research are absolutely necessary for the livestock and poultry industry and for the public health, and these should never be allowed to deteriorate because of programs conducted on the federal level."

Doctor Milligan in his President's Address in 1958 commented, "The combining of the offices of State Veterinarian and Veterinarian in Charge has taken on a new light during the year. This should be viewed with concern by this organization and every other organization interested in animal disease control. These offices have served their separate functions well throughout the years, and have cooperated in most instances without difficulty when working on joint programs. No one can serve two masters well, and the joining of these offices throughout the country can lead only to one end, that is the complete domination and usurpation of state authority by the Federal Government. It is true that at the present time this may seem to be an improvement over some of the states' organizations. These conditions could best be corrected by the states involved. America is not now ready for centralized government in Washington."

Doctor Rosner in his President's Address last year, 1964, ends his remarks addressed to this problem, "It, therefore, behooves us who are Chief Livestock Sanitary Officials of the states concerned to do all within our means and limitations to develop a greater capability at the state level."

Unfortunately all of these remarks were made by men at the termination of their office as president and they were unable to follow up on their thinking as active officers of this Association. This is my position at this, the first opportunity, to formally present my recommendations.

The present shortage of veterinary man power and the prospects of a more critical shortage make it mandatory we correct this waste and duplication in the very near future. We owe this to our nation.

I would suggest that the Animal Health Division, Agricultural Research Service, United States Department of Agriculture, immediately replace their 50 duplicating offices with four regional offices in the United
States. I am certain four regional offices, staffed with exceptionally skilled veterinary medical scientists specializing in various fields of endeavor, could be of invaluable assistance to the states in advising on technical problems, could encourage the development of—instead of depressing and competing against—state staffs, be in an excellent position to coordinate states' activities for the common good, could save untold amounts of money, and would release much veterinary man power away from duplication and waste.

The regional office concept of the administration of federal function is successfully done by many federal agencies which obtain true cooperation with state agencies. The state agencies not only carry out their state functions, but simultaneously accomplish a federal function with no duplication of a myriad of "counter-part" federal employees. There is no reason in the world that this cannot be done for and with the Animal Health Division of the United States Department of Agriculture. The states will cooperate and contribute if not supplanted and if encouraged to do so.

The old argument that a duplicating federal office must be maintained in some states because that state has inadequate staff and facilities to cope with livestock diseases is no longer valid with modern communication and travel. The states will develop adequate staff and facilities if they can no longer let "Uncle" do it and if they no longer have to cooperate with a federal agency to establish such a staff.

The argument that a state failing to function could endanger national welfare is not valid. First affected would be the state welfare and no state would permit a livestock disease to affect its welfare. Secondly, if the disease was of a nature capable of endangering the nation's welfare, the Secretary of Agriculture is vested with extraordinary authority to prevent the spread of that disease. The Secretary of Agriculture should lend emergency assistance through the four regional offices when necessary. This can be done without perpetually maintaining 50 unnecessary duplicating offices.

It is my sincere belief and my sincere recommendation to the United States Livestock Sanitary Association that they no longer ignore Doctor Brandenberg's recommendation. It is imperative you act by creating a top-flight committee to thoroughly and carefully investigate and study duplicity and wastage of veterinary man power in this country and make recommendations to the 70th Annual Meeting.

To the Officers, Chairmen of the Committees, Members of the Committees, and Members of the Association, my personal thanks to each of you for carrying out the tradition of hard work of the United States Livestock Sanitary Association. To the speakers who have come here to share their knowledge with us, please accept my thanks on behalf of the United States Livestock Sanitary Association. May I conclude with the sincere hope that we will always be able to point with pride to the accomplishments of this organization in the service to our homes, states and nation.
PRESENTATION OF PLAQUE AND TIE HOLDER
TO PRESIDENT J. W. SAFFORD

R. A. Hendershott

Trenton, New Jersey

Ladies and Gentlemen in attendance at this our Sixty-ninth Annual Meeting: For the past fifteen years it has been customary to acknowledge the Association's debt to its President for his service to us during his journey through the chairs of second and first Vice-Presidency, President-Elect and finally as President.

This year, in addition to the tie holder made of a replica of the Emblem of the United States Livestock Sanitary Association, we are presenting to Doctor Safford a plaque setting forth the fact that he has in fact received a printed record of his service to us. It occurred to me that few visitors to the office of the retiring President while observing the tie holder, and I trust admiring it, could know the significance of it. If it can be arranged I hope we can provide each past president with a similar plaque in appreciation of their service to this Association.

Doctor Safford, it is my privilege to present these mementoes to you as a token of our appreciation of your service to this Association. In making this presentation we wish you continued health and service as Chief Regulatory Official of Montana and trust you will find a suitable location for the plaque as well as the tie holder.

Dr. John W. Safford: Ladies and Gentlemen: Thank you for this expression of your appreciation of my service as President. I wish to thank the contributors to this our sixty-ninth meeting and all who have assisted in making this meeting the success it bids fair to be.
MEMORIAL SERVICES

Harry E. Goldstein

Mr. President, Members of the Association, Ladies and Gentlemen: Each year it is the custom of our Association to pay respect to our departed colleagues. To the best of our information, the following members have passed away since our last meeting.

Henry D. Bergman (ISU '10) – 79
Ames, Iowa
Died May 20, 1965

Dr. Bergman served as dean of the College of Veterinary Medicine, Iowa State University, and as Director of the University's Veterinary Medical Research Institute from 1943 until his retirement in 1952. He was a past president of the AVMA, the Iowa V.M.A., and the Association of Deans of American Veterinary Colleges.

Dr. Bergman was a fellow of the American Association for the Advancement of Science, a member of Research Workers in Animal Diseases of North America, long a member of USLSA and served on several of our committees.

Cliff D. Carpenter (COR '20) – 68
Sonoma, California
Died August 6, 1965

A leader in the field of poultry veterinary medicine, Dr. Carpenter was meeting with the U. S. Participation Committee of the 13th World's Poultry Congress when he died of a heart attack. He was chairman of the committee, which was finalizing plans for the participation of U. S. veterinarians in the world congress to be held in Russia in 1966.

For the last seven years, Dr. Carpenter was a food consultant for the Cliff D. Carpenter Associates in Sonoma. He formerly served as consultant to the USDA in poultry products, marketing and research. He established the first known private poultry practice in the United States.

He was president of the American Poultry Industry for 14 years, developing a uniform sanitation code for poultry processing plants. He served as chairman of the AVMA's poultry committee, the poultry committee of the USLSA, and the U. S. Ornithosis Committee.

John B. Champlin (ISU '33) – 55
Nashville, Tennessee
Died November 4, 1964

Dr. Champlin was veterinarian in charge in Tennessee for USDA's Agriculture Research Service. Joining the Service in 1934 as field veterinarian in LeMars, Iowa, he later worked in brucellosis laboratories in Georgia, N. Dakota, and Nebraska.
In 1948, Dr. Champlin became assistant veterinarian in charge in Missouri, transferred to Arizona in 1952, and in 1955 assumed the duties of veterinarian in charge in Tennessee. Under his aggressive leadership, Tennessee became modified certified brucellosis free in 1959.

G. Carroll Cilley, Sr. (ONT '26) - 63
Concord, N. H.
Died September 5, 1964

Dr. Cilley was a past-president of New Hampshire V.M.A., and had served as a delegate to AVMA annual meeting. He was a member of the New Hampshire House of Representatives, heading the Merrimac County delegation as chairman for several terms. Dr. Cilley died while on duty as state veterinarian at a race track.

Thomas C. Green (IND '11) - 77
South Charleston, W. Va.
Died March 17, 1965

Dr. Green was West Virginia's first state veterinarian, serving almost a quarter of a century before he retired four years ago. Prior to his appointment as state veterinarian, Dr. Green had engaged in private practice for many years. He had served as president of the U. S. Livestock Sanitary Association and was active in several other professional organizations.

I. Forest Huddleson (MSU '25) - 71
East Lansing, Michigan
Died May 26, 1965

Dr. Huddleson, Professor emeritus of microbiology and public health, Michigan State University, was known worldwide for his basic research on brucellosis. Retiring last year, after 49 years of service at Michigan State University, Dr. Huddleson is generally credited for the procedures which helped bring brucellosis under control. He was the author of 170 professional articles dealing with his research and of three books on brucellosis. He was cited numerous times for the excellence of his research. As a world authority on brucellosis in man and animals, Dr. Huddleson presented many papers from our rostrum.

Erwin L. Jungherr (VI '22) - 68
West Nyack, New Jersey
Died April 16, 1965

Recognized as the world's leading authority on the histopathology of avian diseases, Dr. Jungherr had been head of the Department of Animal Diseases at the University of Connecticut for 29 years before retiring in 1959. The same year he began a second career as pathologist at Lederle Laboratories, performing research on viruses and rickettsia for the company's production of an oral polio vaccine.
Born in Oberndorf, Austria, Dr. Jungherr earned his DVM degree from the University of Vienna, then came to the United States in 1923. Before joining the faculty of the University of Connecticut in 1930, he was assistant professor in the Experimental Station of Montana State College and veterinarian of the Texas Agricultural Experiment Station.

While at Connecticut, he was a collaborator with the USDA, and a special research assistant in the Harvard Medical School. Until his death, he was a consultant to the Armed Forces Institute of Pathology, and chairman of the Committee for Animal Health of the National Research Council. Dr. Jungherr was co-editor of an annual publication, Advances in Veterinary Medicine, and author of more than 100 scientific articles.

Dwight L. Lichty (TEX '44) - 57
West Palm Beach, Florida
Died April 24, 1965

Dr. Lichty had been with the Palm Beach County Health Department since 1950. Following receipt of the M.P.H. degree from Harvard University in 1948, he entered on duty with the Public Health Service and was assigned to the U.S. Communicable Disease Center. During his tour of duty with CDC, Dr. Lichty worked with the Wisconsin State Health Department on a brucellosis field study.

Robert W. Metzger (COR '32) - 55
Syracuse, New York
Died May 8, 1965

Dr. Metzger had been director of quality control for the Dairymen's League Cooperative Association since 1936 and was a nationally recognized leader in his field. He was founder of the National Mastitis Council, serving as its president until this year; past president of the New York State Association of Milk Sanitarians and of the Central New York V.M.A.

A. Stanley Schlingman (OSU '11) - 75
Silver Spring, Maryland
Died October 12, 1964

Before his retirement in 1956, Dr. Schlingman had served 33 years as a veterinarian in the research division of Parke, Davis, & Co. He had been a member of this organization for many years.

Clarence D. Stein (UP '11) - 76
Wilkinsburg, Pennsylvania
Died July 18, 1965

A world-renowned authority on anthrax and infectious anemia, Dr. Stein retired in 1965 after 45 years of public service in research and regulatory work on animal diseases in USDA.

Dr. Stein went to work for the Meat Inspection Branch of USDA in 1911. He served in Virus-Serum Control and Pathological Divisions until
his retirement. During his career, he received many citations for his research on animal diseases, and was the author of more than 40 scientific publications. He appeared on our program some years ago.

James R. Wiley (UP '41) - 46
Myerstown, Pennsylvania
Died December 13, 1964

Dr. Wiley was director of product development and technical services for Whitmoyer Laboratories. Before joining the Laboratories, he operated a private practice. Dr. Wiley was widely known throughout Pennsylvania for his activities on behalf of the poultry industry. He helped organize the state's first diagnostic laboratory, was a past-president of the Pennsylvania Poultry Federation and of the Lebanon Valley Poultry Association. In 1958, he was named the allied poultry industry "Man of the Year."

Long active in civic and church affairs, he held numerous positions of leadership. He was a lifetime member of the U. S. Livestock Sanitary Association.

We respectfully request all present to arise and remain standing to participate in a silent prayer for the peaceful repose of the souls of these deceased members.

SILENT PRAYER

Thank you for your respectful participation.

As we bid farewell to these colleagues who have been called on before, let us be thankful that these men have had the opportunity to so ably contribute to their respective fields, and that we have had the privilege of knowing and working with them. Let us be thankful for the ideals that they have passed on to us and for their contributions to our way of life in a country where men can think, act, and work on their own initiative. It is this type of "idealogy" emplified by our departed friends that needs to be sustained by us who represent the living. The finest tribute we can pay these men is to continue with dedication on the same high plane set by their examples.
REPORT OF THE SECRETARY-TREASURER

Ralph A. Hendershott
Trenton, New Jersey

Ladies and Gentlemen: I am pleased to see so many registrants at our desk for this Sixty-ninth Annual Meeting. As you might have judged from the delay in obtaining copies of the Sixty-eighth Proceedings of the meeting held in Memphis, October 1964, this has been a hectic year for many reasons. I have for several years, asked those who address us from this podium to have two copies of their paper in my hands no later than September 1, prior to the meeting. If we ever achieve this, I will know that the millenium has arrived. At the end of the Memphis meeting I had all but fourteen of the 87 items presented. A few of the missing presentations were promised, mailed to me by Christmas. Many letters and quite a few phone calls were necessary to stimulate the procrastinators to comply. Some I transcribed from the tape recording, which incidentally, was not too good, due to lack of knowledge of the electronics man of his amplifying equipment.

On June 7th, 1965, I received one paper that had finally made it thru the authors review board. On June 13th, I received the second paper from the same source. This later one, incidentally was held over. Two authors promised to send their paper direct to the printer, one did, after a phone call to him, the other failed to send his in, even after a long phone call.

Perhaps these gentlemen do not realize what their action, or rather inaction causes.

First many state legislators are in session and the material contained in our annual report would provide those charged with responsibility, ammunition sorely needed in obtaining favorable action and appropriation for disease control.

Secondly, committee recommendations and reports are an aid to regulatory officials in the development of programs at state level.

Third, research workers could make use of the information contained in the report, but the report looses a great deal of its value if unduly delayed.

Fourth, those who purchase reprints are handicapped by the delay and since many contributors are state or federal employees and most government budgets end on June 30th, it is impossible to get reprints printed, delivered, billed and paid out of the current fiscal year budget.

Believe me, it is of no help to the efficiency of my office.

I trust, therefore, that all who enjoy the use of the podium at this meeting will have the courtesy of leaving a copy of their presentation with the person currently serving as chairman.

I understand that an increasing number of associations are making a charge of up to twenty five dollars, ($25.00) for the privilege of using their podium for presenting ones ideas and research results.
It is odd how extraction of the coin of the realm, stimulates compliance.

Illness prevented me from attending as many of the regional meetings as I would have liked to.

At the president's suggestion, I represented you at the meeting in Chicago, January 18 & 19, 1965, called by Mr. Shuman of the American Farm Bureau. This meeting was held to consider the proposed transfer of meat inspection from the Agricultural Research Service to the new Consumer and Marketing Service of the United States Department of Agriculture. It also dealt with the problem of pesticide and herbicide residues in food of animal origin.

As was anticipated, the disapproval of industry did not prevail. However, several committees were appointed from the organizations present and interested to keep surveillance on future activity and stand ready to recall the group, should the occasion arise.

As usual the meeting of the Committee on State and Federal Relations at Washington, D. C. was attended.

Copies of the presentations made before the budget and program review committee of the United States Department of Agriculture, were mimeographed by our Legislative spokesman, Doctor Ladson and our President-elect, Doctor Campbell and sent to me for mailing to each state veterinarian and members of our Executive Committee and to the head of each state Department of Agriculture. Many complimentary letters were received from recipients of this report.

NEED TO CHANGE THE NAME OF THIS ASSOCIATION

Some few years ago, I presented a request that consideration be given to changing the name of this Association from "Sanitary" to "Animal Health"—either United States Animal Health Association or American Animal Health Association. The need for change has been with us for a long time but was forced to the front as a result of publicity given us at Memphis.

At the 68th Annual Meeting, we achieved our greatest result thus far in publicity and some twelve members appeared on live television and assisted in the preparation of T.V. tapes on various animal diseases. These tapes have been used over and over since that meeting, and the Associations meeting at Memphis reported as the source. I was curious to learn who sponsored this feature and learned that immediately following our presentations a company that sells disinfectant tablets for cesspools and whose name includes the word "Sanitary" put on their sales pitch.

Some years ago I was asked by Texas officials for recommendations concerning their board and I pointed out the desirability of calling it the "Animal Health Division." I am certain the change has been beneficial to them. Every state could profit by the inclusion of the words "Animal Health" in the designation of the activities of their office of animal disease control, that in fact, deals with this subject, so also could this association,
the term is self explanatory and factual. I would recommend that serious consideration be given to changing our Association's name so that the name conveys some idea of our activity.

ACKNOWLEDGEMENT

I wish to thank the officers and members of our Executive Committee, the individual members for their support and the various governmental agencies and their personnel and particularly the chairmen of our committees whose untiring effort and cooperation continues to make this meeting the success that it is. The organization that sponsors the social hour, the Chamber of Commerce of Lansing, the Jack Tar Hotel, and last but by no means least, all those fine contributors who have two copies on my desk by September 1st, and all of you in attendance.

Following is a financial report as prepared by our certified public accountant—I am prepared to explain any item relative to it.

As usual it has been an enjoyable privilege for me to have served you this year.

Mr. Chairman, may I move this presentation following any discussion from the floor be referred to the Executive Committee for their approval.
REPORT OF THE SECRETARY-TREASURER

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR THE PERIOD FROM OCTOBER 1, 1964 TO SEPTEMBER 30, 1965

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash Balance, October 1, 1964:</td>
<td></td>
</tr>
<tr>
<td>First Trenton National Bank, Trenton, N.J.</td>
<td>$ 253.91</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association, Morrisville, Pa.</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association, Albuquerque, New Mexico</td>
<td>5,419.88</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$ 5,674.79</strong></td>
</tr>
<tr>
<td>Increased by Cash Receipts:</td>
<td></td>
</tr>
<tr>
<td>Individual Dues</td>
<td>5,712.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>5,325.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>1,427.00</td>
</tr>
<tr>
<td>Reprints</td>
<td>1,240.54</td>
</tr>
<tr>
<td>Registration Fees</td>
<td>3,610.00</td>
</tr>
<tr>
<td>Brucellosis Facts (What is Known About Brucellosis)</td>
<td>450.00</td>
</tr>
<tr>
<td>Foreign Animal Diseases Handbooks</td>
<td>3,308.69</td>
</tr>
<tr>
<td>Interest on U.S. Treasury Bonds</td>
<td>800.00</td>
</tr>
<tr>
<td>Interest on Sandia Savings and Loan Account</td>
<td>464.58</td>
</tr>
<tr>
<td><strong>Total Cash Receipts</strong></td>
<td><strong>22,337.81</strong></td>
</tr>
<tr>
<td>Decreased by Cash Expenditures:</td>
<td></td>
</tr>
<tr>
<td>Meeting Expenses</td>
<td>799.83</td>
</tr>
<tr>
<td>Printing and Stationery</td>
<td>10,140.80</td>
</tr>
<tr>
<td>Salary</td>
<td>7,500.00</td>
</tr>
<tr>
<td>Communications</td>
<td>788.18</td>
</tr>
<tr>
<td>Travel</td>
<td>1,760.92</td>
</tr>
<tr>
<td>Electricity</td>
<td>108.07</td>
</tr>
<tr>
<td>Rent</td>
<td>360.00</td>
</tr>
<tr>
<td>Insurance</td>
<td>192.60</td>
</tr>
<tr>
<td>Animal Virus Classification (World Health Organization Grant)</td>
<td>1,470.00</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>103.93</td>
</tr>
<tr>
<td>Mastitis Council</td>
<td>200.00</td>
</tr>
<tr>
<td>Conference of Vet. Laboratory Diagnosticians</td>
<td>200.00</td>
</tr>
<tr>
<td><strong>Total Cash Expenditures</strong></td>
<td><strong>23,624.33</strong></td>
</tr>
<tr>
<td>Cash Balance, September 30, 1965:</td>
<td></td>
</tr>
<tr>
<td>First Trenton National Bank, Trenton, N.J.</td>
<td>22.31</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association, Morrisville, Pa.</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association, Albuquerque, New Mexico</td>
<td>4,364.96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$ 4,388.27</strong></td>
</tr>
</tbody>
</table>
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS
FOR THE PERIOD FROM OCTOBER 1, 1964 TO SEPTEMBER 30, 1965

Revenue:
Total Cash Receipts $22,337.81
Accounts Receivable 1,454.00
$23,791.81

Expenditures 23,624.33

Net Revenue from Operations for Fiscal Period $ 167.48

NET WORTH - SEPTEMBER 30, 1965

Accounts Receivable $ 1,454.00
Balance, First Trenton National Bank, Trenton, N.J. 22.31
Balance, Trevose Savings and Loan Association, Morrisville, Pa. 1.00
Balance, Sandia Savings and Loan Association, Albuquerque, New Mexico 4,364.96
U.S. Treasury Bonds, 4% Due February 15, 1980 20,000.00
Furniture and Fixtures 400.00

New Worth, September 30, 1965 $26,242.27

ANALYSIS OF CHANGE IN NET WORTH

New Worth, October 1, 1964 $26,314.79

Increased by:
Net Revenue from Operations for Fiscal Period Ended September 30, 1965 167.48

$26,482.27

Decreased by:
Write-down of Furniture and Fixtures to Realizable Value 240.00

Net Worth, September 30, 1965 $26,242.27
DISEASE CONTROL THROUGH THE STUDY OF POPULATION CHARACTERISTICS

P. R. Schnurrenberger,* D.V.M., M.P.H.; R. J. Martin,** D.V.M.; and P. B. Doby,*** D.V.M.

One of the major obstacles encountered in epidemiological studies of animal diseases is the almost complete lack of information concerning our domestic animal populations. The only available data consist of rough estimates of numbers of the various species with no information on geographic distribution, age, breed, or sex.

Information of this nature would be especially valuable if it could be applied to the determination of high risk herds for the detection of preventable diseases in our cattle population. Disclosure of the characteristics of these herds would enable regulatory officials to concentrate their screening activities on the most productive segments of the population based on a limited geographic area, breed, and herd size.

This report describes a preliminary attempt at obtaining some limited information on the characteristics of the cattle population in one Illinois county then relating these characteristics to the brucellosis status of the animals.

MATERIALS AND METHODS

Pope County, located in Southeastern Illinois just across the Ohio River from Kentucky, was selected for this study for two reasons. Brucellosis testing of all the cattle in the country had recently been completed and the cattle population was sufficiently small to be studied economically. This is a rural county with the center of human population in Golconda, a town of 864. The population of Pope County according to the 1960 census was 4,061.

The data source was the standard brucellosis test record, A.D.E. Form 4-33 (July, 1962), obtained from the Division of Livestock Industry, Illinois Department of Agriculture. These forms were completed by practicing veterinarians during the certification of Pope County from January to May, 1963. These records were transferred to IBM cards for machine tabulation.

**Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia—assigned to the Illinois Department of Public Health.
RESULTS

The most common breed among the 7461 cattle tested was Hereford (4767), followed by Angus (1772), and Holstein (287). There were 164 Shorthorns, 157 Guernseys, 151 Jerseys, 25 Brown Swiss, and 1 Aryshire. Breed was not specified or was designated as mixed for 137 cattle. Thus, 89.8 percent of the cattle were beef or dual purpose breeds while only 8.3 percent were dairy and 1.9 percent unspecified.

Two-thirds of the cattle were six years of age or less (Figure 1). However, 339 of the 392 (86.5 percent) males were in the one to six year age group compared to 66.6 percent of the 6977 females. The four oldest cattle were a 17 year old Angus, 18 year old Hereford, 20 year old Angus, and a 21 year old Jersey. According to the test records, 93.5 percent of the animals were females in contrast to only 5.3 percent males. The sex was not specified for 92 cattle (1.2 percent).

The mean size of the 341 beef herds was 19.7 cattle, nearly twice the 9.4 mean of the 66 dairy herds. This marked difference in herd size is illustrated in Figure 2. The percentage of reactor cattle decreases as the size increases (Table I), while the percentage of herds containing one or more reactors seems to be independent of herd size.

Only 580 cattle were reported as vaccinated (7.8 percent) with the rate among dairy cattle more than triple the rate among beef animals.

Figure 1. Age distribution of 7461 cattle in Pope County, Illinois, 1963.
Figure 2. Size of 341 beef and 66 dairy herds in Pope County, Illinois, 1963.
TABLE I

Brucellosis Reactor Rates by Herd Size, Cattle in Pope County, Illinois, 1963

<table>
<thead>
<tr>
<th>Number of Cattle in Herd</th>
<th>1-10</th>
<th>11-20</th>
<th>21-30</th>
<th>31+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle tested</td>
<td>1147</td>
<td>2000</td>
<td>1732</td>
<td>2582</td>
<td>7461</td>
</tr>
<tr>
<td>Number reactors</td>
<td>15</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Percent reactors</td>
<td>1.31</td>
<td>0.30</td>
<td>0.12</td>
<td>0.08</td>
<td>0.33</td>
</tr>
<tr>
<td>Herds tested</td>
<td>233</td>
<td>134</td>
<td>70</td>
<td>53</td>
<td>490</td>
</tr>
<tr>
<td>Herds with reactor</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Percent with reactor</td>
<td>2.15</td>
<td>1.49</td>
<td>1.43</td>
<td>1.89</td>
<td>1.84</td>
</tr>
</tbody>
</table>

(21.1 percent vs. 6.5 percent). The 11 percent vaccination rate among cattle of unspecified breeds was intermediate between the two. The highest vaccination rate for the cattle of known age was 21.7 percent among those less than one year of age, however a rate of 37.9 percent existed among the 132 cattle of unspecified age (Table II).

The rates of both suspect and reactor cattle were significantly (P<0.05) higher among the dairy breeds (Table III) and among the cattle 1-10 years of age (Table IV).

TABLE II

Age-Specific Brucellosis Vaccination Rates Among Cattle from Pope County, Illinois, 1963

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>&lt;1</th>
<th>1-5</th>
<th>6-10</th>
<th>11+</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Cattle</td>
<td>594</td>
<td>4205</td>
<td>2291</td>
<td>239</td>
<td>132</td>
<td>7461</td>
</tr>
<tr>
<td>No. Vaccinated</td>
<td>129</td>
<td>267</td>
<td>116</td>
<td>18</td>
<td>50</td>
<td>580</td>
</tr>
<tr>
<td>Percent Vaccinated</td>
<td>21.7</td>
<td>6.3</td>
<td>5.6</td>
<td>7.5</td>
<td>37.9</td>
<td>7.8</td>
</tr>
</tbody>
</table>

TABLE III

Breed-Specific Brucellosis Reactor and Suspect Rates in Pope County, Illinois, 1963

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. Cattle</th>
<th>No. Reactors</th>
<th>Percent Reactors</th>
<th>No. Suspects</th>
<th>Percent Suspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereford</td>
<td>4767</td>
<td>12</td>
<td>0.25</td>
<td>58</td>
<td>1.22</td>
</tr>
<tr>
<td>Angus</td>
<td>1772</td>
<td>7</td>
<td>0.39</td>
<td>12</td>
<td>0.68</td>
</tr>
<tr>
<td>Shorthorn</td>
<td>164</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>0.61</td>
</tr>
<tr>
<td>Holstein</td>
<td>287</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>1.39</td>
</tr>
<tr>
<td>Guernsey</td>
<td>157</td>
<td>2</td>
<td>1.28</td>
<td>4</td>
<td>2.55</td>
</tr>
<tr>
<td>Jersey</td>
<td>151</td>
<td>2</td>
<td>1.32</td>
<td>22</td>
<td>1.32</td>
</tr>
<tr>
<td>Brown Swiss</td>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Unknown</td>
<td>137</td>
<td>2</td>
<td>1.46</td>
<td>2</td>
<td>1.46</td>
</tr>
<tr>
<td>Total</td>
<td>7461</td>
<td>25</td>
<td>0.33</td>
<td>83</td>
<td>1.11</td>
</tr>
</tbody>
</table>
TABLE IV
Age-Specific Reactor and Suspect Rates for Brucellosis in Cattle from Pope County, Illinois, 1963

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>&lt;1</th>
<th>1-5</th>
<th>6-10</th>
<th>11+</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Cattle</td>
<td>594</td>
<td>4205</td>
<td>2291</td>
<td>239</td>
<td>132</td>
<td>7461</td>
</tr>
<tr>
<td>No. Reactors</td>
<td>—</td>
<td>12</td>
<td>12</td>
<td>—</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Percent Reactors</td>
<td>0.0</td>
<td>0.3</td>
<td>0.5</td>
<td>0.0</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>No. Suspects</td>
<td>—</td>
<td>48</td>
<td>32</td>
<td>1</td>
<td>2</td>
<td>83</td>
</tr>
<tr>
<td>Percent Suspects</td>
<td>0.0</td>
<td>1.1</td>
<td>1.4</td>
<td>0.4</td>
<td>1.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The suspect rate of 0.3 percent among the vaccinated cattle (2/580) was approximately one-fourth the 1.2 percent (81/6881) found among the non-vaccinated cattle. No reactors were found among the vaccinated cattle.

DISCUSSION

The source of information introduced a major bias into this study since cattle less than six months of age and steers are ordinarily not tested for brucellosis. It is to be expected, therefore, that the stated population characteristics are inaccurate in the less than one year age group and in the sex analysis.

Further inaccuracies are introduced by the fact that the forms were completed in the field at the time blood specimens are collected and were intended to serve only for the identification of individual animals if they should be suspects or reactors. Therefore, in many instances, the forms were not completed fully and the ages may have been estimated.

The small number of reactor animals further complicates interpretation of the data.

The final point in qualifying these data is that while Pope County might be fairly representative of Southern Illinois, it is not possible to transpose the information to other portions of the state or nation. This report is intended to serve as a model for an approach to disease control not as a definitive statement on the cattle population of the country.

Expressed in terms of number of reactor per cattle tested, the most productive group for testing is the unvaccinated dairy animals five years of age or under. The reactor rate in this group is 0.97 percent (4/413) in contrast to 0.28 percent (20/7048) among all other animals. This is such a small segment of the cattle population of Pope County, however, that the approach would be impractical.

A more realistic technique would be to test all herds of twenty animals or less. Using this method, it would have been possible to find 83 percent of the reactors by testing only 42 percent of the animals. The advantage is less obvious when analyzed on a herd basis, for 60 percent of the 490 herds would have been tested to find seven of the nine reactor herds. Two of the undisclosed reactors would have been in a herd of 26-30
animals while the other two were in a herd of over 40. Certainly the disease transmission potential of a reactor is much greater in a herd of 40 than in a herd of ten.

The disadvantages of the "high-risk" approach described here, are obvious in the eradication phase of a disease control program such as brucellosis. In this final phase it is imperative to find every infected animal. On the other hand, this approach could be extremely valuable in the early stages of the control of a disease when the immediate objective is to rapidly locate the greatest possible number of infected animals with the smallest amount of test effort.

SUMMARY

Using brucellosis test records, a cattle census was performed in Pope County, Illinois. Nearly 90 percent of the cattle tested were beef breeds. Two-thirds of the cattle were six years of age or less. The beef herds averaged nearly twice the size of the dairy herds.

Reactor rates were higher in small herds, dairy breeds, animals under 10 years of age and unvaccinated cattle.

The value of this approach in disease control is discussed.
REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

F. D. Maurer, College Station, Texas, Chairman; D. E. De Tray, Beltsville, Maryland, F. P. Gluckstein, Alexandria, Virginia; N. Konnerup, Silver Spring, Maryland; K. L. Kuttler, Kikuyu, Kenya; N. L. Meyer, Hyattsville, Maryland; G. Poppensiek, Ithaca, New York; F. K. Ramsey, Ames, Iowa; R. C. Reisinger, Hyattsville, Maryland; F. A. Todd, Arlington, Virginia; H. G. Wixom, Sacramento, California

The years 1964-1965 have been marked by some significant developments in the world situation regarding exotic diseases.

Rinderpest:

The massive rinderpest control project in West Africa in which millions of animals (in some areas more than 80 percent of the population) have been immunized is a good example. The sharp reduction in foci of infection resulting from the program has encouraged other countries in this area of the world to set up similar programs. These programs provide hope for the possible elimination of this disease. At the same time, however, optimism should be tempered with the possibility of wildlife reservoirs permanently perpetuating the disease. It should be noted that after ten years of freedom from the disease, rinderpest was re-introduced to the Arabian Peninsula apparently from Somalia.

Foot & Mouth Disease:

The FMD problems remain serious, even critical in many areas of the world. Extension of SAT 1 into parts of Asia complicate control efforts in that area and continues to pose a threat to Europe. A variant Type A to which conventional A vaccines do not confer immunity was identified in the Middle East by Israeli workers.

During the last half of 1964, a marked increase in FMD, mainly in pigs, occurred in many areas of Europe. The position in the Americas remained essentially stable except for an outbreak of Type O in Guadalupe. Argentina expanded its vaccination program significantly with a goal to reach 95 percent coverage.

African Swine Fever:

In early 1964, African swine fever occurred on 11 farms in France. It has apparently been eliminated. Intensive eradication of swine showing signs characteristic of either hog cholera or ASF is carried out in the Spanish border areas. The disease remains active in the Iberian peninsula and work at Plum Island indicated the virus obtained from that area is equally as virulent as the Spencer strain from Africa when inoculated into American Swine.

Spanish workers have incriminated a tick as a potential vector. The significance of this finding in the natural transmission of the disease
should be considered with reservation. Swine husbandry practice in Af-rica requires double fencing of pig paddocks which excludes contact with wild porcine species but the practice does not necessarily exclude the transfer of ticks.

_Equine Infectious Anemia:_

Equine infectious anemia was reported for the first time in Hong Kong and Argentina. Mycoplasma infection, producing pneumonia in sheep, was diagnosed in Argentina. Mycoplasma organisms were isolated from a large herd of goats in Mexico in which losses were extensive from pneu-monia.

_Contagious Bovine Pleuro-pneumonia:_

CBPP is still a critical problem in many areas. The disease has re-appeared in Nigeria and other parts of West Africa where it had once been sharply reduced or virtually eliminated. Research has produced more ef-fective diagnostic techniques and effective artificial transmission meth-ods. The uncertainties regarding vaccine efficacy and administration remain a problem.

_Arbor Viruses:_

Information on the arthropod borne virus diseases continues to de-vlop at a rapid rate and individual evaluation of the information keeps this broad spectrum of virus diseases in a state of flux. New viruses are continuously uncovered and their significance to hosts and vectors outside known circumscribed ecological habitats remain obscure. This situation also contributes to the problems of virus classification. National and in-ternational committees on classification and nomenclature of viral agents are working to clarify the picture. These committees are mentioned here to alert the membership of forthcoming changes.

_United States Livestock Sanitary Association Foreign Disease Book:_

The second edition of "Foreign Animal Diseases" originally published in 1954 was produced this year. It is the opinion of the committee that such a handbook or manual should be periodically revised. The schedule for revision should not exceed five years and probably not be undertaken at less than three year intervals. This 300 page book covering 21 diseases may be obtained from the Secretary of the United States Livestock Sanitary Association.

_Research:_

A need for continuing research on foreign animal diseases is recog-nized by the committee. Several nations where foreign animal diseases are enzootic have adequate laboratory facilities and competent technical personnel to conduct research. Some of them are also well endowed with Public Law 480 funds to finance the research. The Committee
recommends that the Agricultural Research Service determine countries of choice for this work and offer technical guidance to the nations selected.

**Canadian Quarantine Station:**

In January 1965 the Canadian Department of Agriculture announced a plan to establish a maximum security quarantine station to import cattle from foot-and-mouth disease countries. The station is under construction and permits have been issued to livestock men to import approximately 125 animals in the fall of 1965. To be eligible for entry under the Canadian plan, animals must be under nine months of age and unvaccinated for FMD. The import procedures require a minimum of seven months of quarantine and a minimum of three serological tests for FMD in addition to tests for other animal diseases. All tests, quarantines and movements of animals will be under the direct supervision and control of veterinarians employed by the Canadian Health of Animals Division. United States Department of Agriculture scientists have evaluated the Canadian procedure and agree that they are sound and will not be a means of introducing foreign diseases, and therefore no additional health requirements are necessary for cattle entering the United States from Canada.

**Experimental Animals:**

In 1964-65 there has been a notable increase in the importation of laboratory animals. Their entry, transportation and use in this country need continuing evaluation as potential means of introducing exotic diseases.

There is accumulating evidence of the importance of small feral animals, and possibly reptiles and amphibians, as reservoirs of human and animal pathogens, particularly arboviruses and protozoan parasites.

For example, the rodent, *Calomus callosus*, has been incriminated as being perhaps the main carrier of Bolivian hemorrhagic fever, a highly virulent agent for humans and perhaps other animals. Studies in Panama have shown that cotton rats, herons, chicoa birds, lizards and other species may serve as reservoirs of infection in maintaining the cycle of Venezuelan equine encephalitis (VEE) virus. The *spiney legged pocket mouse* has been shown to be a host in the jungle to *Leishmania braziliensis*, along with *tree, spotted, and brown rats* and probably many other rodents. Garter snakes have been shown to be capable of remaining infected for long periods of time with Eastern Equine Encephalitis (EEE) virus.

Some animals from "plague" areas may be infested with vectors of *Pasteurella pestis*—others may be harboring *Boophilus annulatus* or other exotic arthropods which can be vectors of significantly economic animal diseases.

It is also noted that the fowl tick, *Argus persicus*, probably the most universal of ticks in its distribution, is present on most fowl importations, particularly the larger wild birds which are in the habit of roosting on the larger branches of trees, for example herons, guinea fowl and hededyah ibis. These ticks may be the vectors of various animal diseases and
means should be developed and exercised for their positive elimination from wild and domestic fowl imported into this country.

Further attention must be given to practical importation regulations with regard to rodents, primates, birds, reptiles, and amphibians as well as the vectors which these species may harbor. For some diseases particularly hemoproteozoon diseases it may prove more practical to control the entry of vectors than to detect animals which are often inapparent carriers of infection.

Therefore, a prior permit for entry of rodents and other small mammals captured in the feral (wild) state is required by the Animal Health Division, Agricultural Research Service, United States Department of Agriculture, or by the Foreign Quarantine Division, United States Public Health Service.

**Etiologic Agents:**

The Committee recognizes the expanding biological research activity in this country and notes the greatly increased use of exotic organisms; fowl plague, influenza strains of animal origin and particularly arthropod-borne viruses. Many of these viruses are of animal disease as well as human disease significance and are potentially capable of becoming established in this country. The occurrence of many accidental infections, overt and sub-clinical, in laboratory personnel has been documented. Some infections, even sub-clinical ones, are accompanied by a viremia of several days duration. A person so infected can be the primary source of establishing a man—mosquito—bird—mosquito—animal cycle.

Many workers using these agents are unaware of their ecological potentialities. Once an agent escapes from the laboratory it may be a long while before it is reisolated as the organism follows a natural cycle. By then, it may have done great damage and it will often be impossible to trace its origin.

United States Department of Agriculture and United States Public Health Service regulations prohibit importation into the United States of such organisms except under conditions and for purposes specified in a prior permit. However, the Committee is increasingly aware that many exotic organisms, and vectors, have purposely or inadvertently been introduced into this country without prior permit and are being used in some cases for purposes and under conditions which are unduly hazardous to human and animal health, and to the general economy.

It is recommended that thought be given for a cooperative effort involving state and federal veterinary and public health officials to canvass all teaching, research diagnostic and commercial laboratories in each state to determine and list the locations of all exotic organisms and vectors and the conditions under which and purposes for which they are being used. Following location of these exotic agents, their handlings, retention and distribution should be controlled to prevent their being hazards to human and animal health.
REPORT OF THE COMMITTEE ON MASTITIS

E. J. Kersting, Storrs, Connecticut, Chairman; R. K. Anderson, St. Paul, Minnesota; H. S. Bryan, Kalamazoo, Michigan; J. H. Drayer, Columbus, Ohio; G. E. Morse, Kennett Square, Pennsylvania; K. J. Peterson, Corvallis, Oregon; R. J. Schroeder, South Gate, California; J. V. Smith, Hartford, Connecticut

The Mastitis Committee met from two to four p.m. on Tuesday, October 26, 1965, in Room 501 at the Jack Tar Hotel. Present at the open meeting were Doctors Wineland of Purdue, Garlick of Hyatsville, Fichandler and Daniels of Connecticut, Bearden of Mississippi State and Beck of Michigan State. The chairman was the only official member of the committee present.

While this report does not reflect the opinions of the official Mastitis Committee, it does represent the interest in, and concern for mastitis, of the men whose names I read; men with backgrounds in teaching, research, extension and regulatory activities.

Recommendations offered for your consideration are as follows:

1. That the United States Livestock Sanitary Association Mastitis Committee should continue to function as a standing committee. Its position should be one of coordination with the National Mastitis Council; and in addition, it should consider certain responsibilities which might not be covered by the Council.

2. That the United States Livestock Sanitary Association Mastitis Committee members be appointed on a staggered term basis in order that continuity of committee activity be maintained. Consideration might be given to three-year terms and to the selection of the chairman from the committee.

3. That the United States Livestock Sanitary Association subscribe to membership in the National Mastitis Council with the payment of $200 annual dues.

4. That the Mastitis Committee be charged with the task of considering and developing, if necessary, minimum standard procedures for helping the dairyman control mastitis at the farm level.
The Committee on Meat and Milk Hygiene recommends that the United States Livestock Sanitary Association urge each state that has not already done so to enact appropriate legislation, and/or promulgate adequate regulations to control the movement of dead, dying, diseased and crippled animals; to prevent the use of dead and dying animals for food and to require their proper destruction; and to condemn and require destruction in whole or in part of those diseased and crippled animals determined by thorough ante mortem and post mortem inspection to be unfit for human consumption.

The Committee will continue to furnish the maximum assistance and support to State Meat and Poultry Inspection Programs. During the past year such assistance was provided to more than twenty-five states desiring either to establish new statewide programs or expand, strengthen or modernize those in existence. Such support consisted of furnishing administrative and technical material, visitations by and consultations with Committee Members, review of proposed laws and regulations, appearance on programs of state, national and international Veterinary Medical Association Meetings, training of Veterinary and other meat inspection personnel and preparation of papers on topics covering various aspects of meat and poultry inspection.

The responsibility of the Veterinary Profession to the public interest in providing professional competence and leadership in meat and poultry inspection programs is internationally accepted. Such programs are designed to prevent the spread of animal disease to men and assure that animals and animal products are handled and processed in accordance with modern sanitary concepts and standards of common decency. Your Committee recommends that the United States Livestock Sanitary Association again endorse and support the resolution passed by the House of Delegates of the American Veterinary Medical Association at its 100th Annual Meeting in New York City in 1963. That Resolution—published in the 1964 Report of this Committee—encourages each State Veterinary Medical Association "to establish an active food inspection Committee to be charged with the responsibility of keeping abreast of meat and poultry inspection developments within the state."

The Model Meat and Poultry Inspection Law prepared by the Meat Inspection Division, United States Department of Agriculture is being
revised by this Committee to strengthen its provisions. The revised version will be presented prior to January 1, 1966 for consideration and approval of the Meat Inspection Division, United States Department of Agriculture, the Executive Committee of the United States Livestock Sanitary Association and the Council on Public Health and Regulatory Veterinary Medicine, American Veterinary Medical Association. The revision will be available for use of the Council on State Governments and other interested agencies and individuals as a guide in preparing a state meat and poultry inspection law.

The Committee has reevaluated its responsibilities to the Association and based on considered judgement is presently organizing itself into subcommittees to study in depth the following eight major areas:

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 Extend Federal Meat Inspection. (Senate #2678 and HR #11670 - 89th Congress of U.S.)
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.

Papers have been prepared and presented by members of this Committee at the meetings stated below:


At the 4th Biennial Symposium, World Association of Veterinary Food Hygienists, Lincoln, Nebraska, July 25 - 30, 1965, the following three papers:

"The Development of a Modern Meat Inspection Program" by Wm. E. Jennings, Albany, New York.

"The Training of Supervising Veterinary and Law Food Hygienists" by Wm. E. Jennings, Albany, New York.

"Trichinosis, Botulism, Staphylococcal Food Poisoning; Prevention and Control" by P. J. Brandly, Washington, D. C.

The official in charge of State Meat Inspection Programs in each state 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 Meat Inspection Division, United States Department of Agriculture at four
regional conferences in San Francisco, Atlanta, Philadelphia and Chicago during March and April 1965. These two day conferences, sponsored by the Secretary of Agriculture, Washington, D.C., will be held again in the Spring 1966. The Secretary of Agriculture is to be commended for his foresight in supporting such conferences which foster effective Federal-State relations in Meat, Milk and Poultry Inspection Programs. Your Committee recommends again that each state collaborator appointed as the Official Collaborator with the Meat Inspection Division, be reevaluated by his state and in consonance with his duties, responsibilities and capabilities in a Total modern meat inspection program.

Increased participation in programs of regulatory, public health and veterinary organizations at local, regional, state, national and international levels is urged by this Committee. Members of this Committee and others qualified by training and experience in meat, milk and poultry technologies are enjoined to foster the efforts of the Veterinary Profession in discharging its responsibilities in insuring a safe, wholesome, unadulterated food supply.

Appropriate liaison is being established and will be maintained with the Council on Public Health and Regulatory Veterinary Medicine of the American Veterinary Medical Association, the National Association of State Departments of Agriculture and other committees of the United States Livestock Sanitary Association, in matters relating to meat, milk and poultry inspection and other interests of mutual concern. Such liaison will assist this Committee in achieving its stated objectives. The Committee was represented by two of its members at a joint meeting of the Executive Committee of the National Association of State Departments of Agriculture and official representatives of the Secretary of Agriculture in Washington, D.C. on July 22, 1965. This meeting, sponsored by the Secretary of Agriculture, considered the aspects and implications of the Bill amending the Federal Meat Inspection Act and extending Federal Meat Inspection.

As official representative of this Association, the Chairman of this Committee attended the 4th Biennial Symposium of the World Association of Veterinary Food Hygienists, sponsored by a Rockefeller Grant and held at the Center for Continuing Education, University of Nebraska, Lincoln, Nebraska July 25-30, 1965. Representatives of 34 countries were in attendance. The literary program included many excellent presentations on topics of worldwide significance in food hygiene. The Organizing Committee and the Nebraska Center of Continuing Education are commended for the excellency of the programming and the facilities provided for that Symposium.

A meeting of Teachers of Food Hygiene in the Colleges of Veterinary Medicine in the United States and Canada was held in Lincoln, Nebraska on July 30, 1965, with members of this Committee assisting in the deliberations. Activities of the United States Livestock Sanitary Association in meat, milk and poultry inspection were presented to assist in further strengthening the instruction in food hygiene in the Colleges of Veterinary Medicine.
The Office of Education, Department of Health, Education and Welfare, Washington, D.C. has requested and received assistance in development of a modern course for the training of food technologists in Institutions of Higher Learning. Consultation and assistance has been furnished as requested to several universities and Agriculture and Technical Colleges desiring to establish courses in food technology. Active participation in the instruction in meat hygiene in four technical colleges has been accomplished during the past year. The Committee is of the opinion that such assistance will provide personnel trained in the fundamental concepts of meat, milk and poultry hygiene and inspection. Potential meat, dairy and poultry inspectors are presently in critically short supply. It is, indeed, fortunate that several colleges and universities recognize this need and have taken appropriate action to develop courses to train personnel for this specialized work.

Bills—Senate - 2678 and HR - 11670—were introduced into the 89th Congress of the United States on October 19, 1965. These bills have been developed from a Compendium of Ideas prepared by a Task Force of the United States Department of Agriculture, headed by Assistant Secretary of Agriculture, Honorable George L. Mehren. This Committee has not had sufficient time to study and evaluate the many facets of this proposed legislation. For this reason, your Committee is unable at this time to commit itself to a stand on this proposal. Immediate, intensified study of this proposed legislation will be accomplished by this Committee to permit furnishing appropriate comment and recommendations if and when requested by the Executive Committee of the United States Livestock Sanitary Association.

A continuing study of humane slaughter is being made with appropriate recommendations to all interested agencies and individuals.

A continuing study and evaluation of State Meat, Milk and Poultry Programs has been initiated. This comparative study will be intensified and expedited to permit this Committee to make appropriate recommendations in these three respective programs of the 50 states. Simultaneously, your Committee will continue to furnish assistance and guidance when requested by any state. Active liaison will insure such assistance.

This Committee is of the opinion that prerequisites for employment of meat and poultry inspectors are not satisfactory. To this end the Committee has consulted with the Professional Testing Service of the American Public Health Association to develop a suitable examination for selection of personnel. This project is receiving the enthusiastic support of that Testing Service. Your Committee is conducting a critical review of present employee selection procedures in an effort to improve meat and poultry inspection in the United States. It is recommended that states and Agencies employing meat, milk and poultry inspectors adopt the policy of selecting only those who have completed satisfactorily one year of college.

The Committee further recommends that a pre-employment physical examination be conducted and that such examination include a determination of the candidate's ability to meet specific criteria relative to environmental and functional factors inherent in the employment as a meat, milk and poultry inspector.
Immediately subsequent to employment the new appointee should receive not less than five weeks of intensive formal (20 percent) and applicatory (80 percent) training. A minimum probationary period of six months subsequent to appointment is recommended.

Employment of Veterinary Meat, Milk and Poultry Inspectors should be restricted to graduates of Veterinary Colleges in the United States and Canada accredited by the American Veterinary Medical Association and to graduates of Veterinary Colleges in other countries recognized by the American Veterinary Medical Association. A nine week training period—formal and applicatory—is recommended for each new Veterinary appointee.

Additional training for Meat, Milk and Poultry Inspectors and Veterinary Inspectors should be provided as required to enhance their competence and promote uniformity in the inspection program.

The Committee recommends that the benefits of meat and 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 endorse all aspects of a Total meat and poultry inspection program. Such programs should be staffed with properly selected personnel, properly remunerated and free of conflict of interest and other influence. The program should include adequate control of construction and sanitation, ante mortem and post mortem inspections, label evaluation and approval, inspection during processing and effective safeguards to prevent the entry into food channels of unfit or unwholesome meat and meat food products.
REPORT OF THE REPRESENTATIVES TO THE ANNUAL MEETING
OF THE NATIONAL ASSOCIATION OF STATE
DEPARTMENTS OF AGRICULTURE

Dr. Grant S. Kaley, Albany, New York, Representative; Dr. Edwin L. Brower, Trenton, New Jersey, Representative; Dr. J. W. Safford, Helena, Montana, Alternate; Dr. R. A. Hendershott, Trenton, New Jersey

Dr. G. S. Kaley and Dr. E. L. Brower, official representatives of this Association to the annual meeting of the National Association of State Departments of Agriculture attended a five-day session in Princeton, New Jersey. Dr. R. A. Hendershott, our secretary, Dr. F. J. Mulhern, Director, Animal Health Division, Agricultural Research Service, Washington, D.C. and Dr. G. C. Stiles, State Veterinarian of Missouri were also in attendance. We met with the Animal Health Committee where a full and frank discussion of proposed resolutions was held. All courtesies were extended to the representatives of this Association with full participation in the discussions.

Seven resolutions were presented to the Animal Health Committee of which two were adopted. Donald L. McDowell, Director, Wisconsin Department of Agriculture is chairman and Lewis Meibergen, President, Oklahoma Board of Agriculture is vice-chairman.

The following resolutions were adopted:

Animal Health

BOVINE MASTITIS

WHEREAS, bovine mastitis is of great economic importance to dairy farmers and is of concern to all segments of the dairy industry; and

WHEREAS, estimates based on figures from the United States Department of Agriculture Statistical Reporting Service indicate an annual loss of more than four hundred million dollars for the period 1951-60 representing loss of animals and milk, not including the cost of treatments; and

WHEREAS, there has been a general lack of progress in mastitis control; and

WHEREAS, it has been demonstrated that an effective control program based on education, herd management, sanitation, proper use of equipment and treatment of affected animals is beneficial to the dairy industry; therefore

BE IT RESOLVED, that the National Association of State Departments of Agriculture in convention assembled in Princeton, New Jersey, October 3-7, 1965, request the United States Department of Agriculture to
immediately intensify work with the State Departments of Agriculture in the development of a proposed National Mastitis Program.

**BRUCELLOSIS INDEMNITIES**

WHEREAS, the experience of animal disease control officials engaged in bovine brucellosis control work discloses that in herds badly infected with brucellosis, it is advisable from the standpoint of both effective eradication and government economy to condemn nonreactor cattle in such herds; the condemnations of such nonreactor cattle being based upon the herd history, current tests, and the best professional judgment of the responsible state and federal officials in the state in which the herd is located; and

WHEREAS, the bovine brucellosis programs of some of the states now authorize the classification of such nonreactor cattle as affected with brucellosis upon the basis of sound professional judgment, along with the payment of state indemnity monies to the cattle owner; however, matching federal indemnity payments in such instances are not authorized; therefore

BE IT RESOLVED, that the National Association of State Departments of Agriculture, in convention assembled in Princeton, New Jersey, October 3-7, 1965, requests its Executive Committee to confer with proper officials of the United States Department of Agriculture on the feasibility of effecting such changes in the federal brucellosis program as to permit the payment of indemnities on condemned cattle in herds badly infected with brucellosis even though individual animals do not react to a brucellosis test.

The following resolutions were presented, but it was thought there was no need for any action by the National Association of State Departments of Agriculture:

**Hog Cholera.** The National Association believes that good progress is being made in the eradication of hog cholera and that previous resolutions were sufficient.

**Brucellosis Calfhood Vaccination.** Again it was thought not necessary to resolve, as the gradual de-emphasis of calfhood vaccination depends largely on area and at this time more study and education are needed.

**Movement of Livestock.** The identification of livestock moved interstate needs more study and development. As this is being done by the Agricultural Research Service and a committee of the United States Livestock Sanitary Association, the National Association did not feel that a resolution was necessary.

**Importation of Livestock From Quarantined Areas.** This was in regard to the importation of cattle from foot-and-mouth countries to Canada. Believing that the Agricultural Research Service and the Canadian
Government have implemented a very sound plan for the importation of calves from these areas, it was not deemed necessary to resolute on this matter.

Sale Yard Inspection of Approved Yards. Feeling that there was more study needed and that again the Agricultural Research Service and the committees of the United States Livestock Sanitary Association were cognizant of this problem, it was thought that a resolution was not necessary.

Dr. W. L. Bendix of Virginia attended the meeting as a representative of the Dairy Committee of the Association. He met with the Dairy, Food and Drug Committee and the following resolution on meat inspection was adopted:

WHEREAS, it is recognized that there exists a need to update present meat inspection laws—both state and national and it is also recognized that there also exists a need for cooperative state-federal meat inspection programs designed to adequately safeguard the public interest; and

WHEREAS, in the development and implementation of such a program it is important that every effort be made to avoid duplication of such procedures as licensing, bonding, inspections, quality control, and all other functions involved in a total meat inspection program; and

WHEREAS, under any such a cooperative state-federal program a procedure for continuous evaluation of program activities by both parties jointly must be established and maintained; therefore

BE IT RESOLVED, that the National Association of State Departments of Agriculture in convention assembled in Princeton, New Jersey, October 3-7, 1965, recommends

I. That a truly cooperative state-federal meat inspection program be developed, including proper legislative action including adequate financing at both levels.

II. That the program so developed be on a fully cost sharing basis.

III. That the program must provide for a transition period of sufficient length to permit state governments and industry to meet agreed upon standards on a graduated time basis.

IV. In order to meet such agreed upon standards a state-federal committee be established jointly by the United States Department of Agriculture and the Executive Committee of the National Association of State Departments of Agriculture to evaluate the program as to transition time or times, adequate financing, responsible supervision, qualified personnel, and all other pertinent functions necessary to a total meat inspection program.

The representatives of the United States Livestock Sanitary Association attended all of the open sessions and the various tours that were planned.

The next meeting of the National Association States Department of Agriculture will be held in Hawaii.
REPORT OF THE COMMITTEE ON NOMINATIONS

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


We congratulate the membership in their selection of Dr. C. L. Campbell as President-elect, and the Committee is proud to place his name in nomination as President. For President-elect, Grant S. Kaley, Albany, New York. For Vice-President, John H. Quinn. And for Second Vice-President, John O'Harra, Reno, Nevada. Mr. President, this is the selection of the Committee.

VOICE: I move the nominations for President be closed. Second.

CHAIRMAN: The motion has been made that the nominations be closed, and the President be elected by acclamation. Are you ready for the question. All those in favor, aye. All those opposed, say no. Dr. M. D. Mitchell makes a motion that the nominations be closed and the President-elect be elected by acclamation. It's been seconded. All those in favor, say "aye." Carried, Dr. Kaley elected President-elect. Is there any further nominations for the office of First-Vice-President?

VOICE: I move that the nominations for office of First-Vice-President be closed and that Doctor Quinn be elected by acclamation. Seconded—

CHAIRMAN: All in favor signify by aye. Opposed. I declare Doctor John H. Quinn elected. Next is the office of Second-Vice-President. Are there any further nominations?

VOICE: I move the nomination for Second-Vice-President be closed and that Dr. John L. O'Harra be elected by acclamation.

CHAIRMAN: Seconded. All in favor of the motion please so indicate. The ayes have it. Doctor O'Harra, you are elected.

It's been moved and seconded that the regional delegates be elected as presented by the Nomination Committee. All those in favor of the motion, signify by saying aye. Those opposed? The regional delegates as listed have been elected.

Will someone escort each of the elected members to the rostrum?
CHAIRMAN: Doctor H'Harra, do you wish to address the Assembly?

DR. J. L. O'HARRA: Thank you for electing me an Officer of this Association, it is certainly a privilege; it also carries a great responsibility. I thank each of you for the privilege and I'll do my very best to discharge the responsibility. Thank you.

CHAIRMAN: Doctor Kaley, it is an honor to have you elected. Would you like to say a few words?

DR. G. S. KALEY: Well, thank you very much, gentlemen; I always say that I'll do the very best I can to carry out the wishes and purpose of this organization.

CHAIRMAN: Dr. Quinn, you are our First-Vice-President and perhaps you would like to say a few words.

DR. J. H. QUINN: I want to convey my sincere appreciation of my progress through the chairs of this organization, and I am coming to realize what a tremendous responsibility the President is faced with each year. However, even with that to look forward to I am very much in appreciation and eager to go on with this,—and in addition, and in conclusion, on behalf of Michigan Agriculture and the Michigan Department of Agriculture I want to say that we were sincerely honored that this organization would bless us with meeting in our State this year. We are happy to have had you—we only hope that you have all had a good stay in our City of Lansing, Michigan, this week. Thank you very much.

CHAIRMAN: Dr. Wixom, could you escort Dr. Campbell, your President for the coming year.

DR. JOHN SAFFORD: A big burden has been handed over to Dr. Campbell; I think you have been very wise and suggest that you have elected a very capable individual and we are looking forward to great things from him. So, it's with a great deal of pleasure that I turn over the gavel to Dr. Campbell.

DR. CAMPBELL: I have made it a practice during this meeting to keep things as short as possible and I think I may have been accused of ramrodding a lot of things through the Executive Committee Meeting. However, I do wish to make this statement, which also will be brief. I feel, John, that you have done a most successful job in guiding the affairs of this Association this year. I only hope that I can do half as well. Thank you.

I was just going to say, John, you already have turned some of your work over to me. I wonder if the regional delegates are in the room. Mr. Timm? Doctor Bodenweiser, will you escort Mr. Timm forward. Mr. Archie Wilson? Is Archie in the room? Mr. Finley, he came in this morning, is he here? Mr. Bishop? Is Mr. Bishop in the room? Mr. Van Horn? Dr. Henning? Dr. Henning has left. It looks like you're standing alone up here, Mr. Timm, but allow me to congratulate you upon your selection as one of the regional delegates. Mr. Timm, would you care to say a few words?
MR. TIMM: Frankly, I think this is superfluous; it's been a pleasure and also a privilege to be a delegate to this organization no matter what name it assumes. I still think it's a privilege and an honor to serve as a Representative of industry to this organization.

CHAIRMAN: Any further business? If not, the 69th Annual Meeting of the United States Livestock Sanitary Association is hereby adjourned.
CONSTITUTION AND BY-LAWS

OF THE

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

ARTICLE I—NAME

The name of this Association shall be "The United States Livestock Sanitary Association."

ARTICLE II—PURPOSE

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

ARTICLE III—MEMBERSHIP

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

OFFICIAL MEMBERSHIP

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

INDIVIDUAL MEMBERSHIP

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

JUNIOR NON-VOTING MEMBERSHIP

Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4-H Club member as well as future farmers under 21 years of age are eligible to election as junior non-voting members.
ARTICLE IV—MEETINGS
The meetings of this Association shall be annual and special.

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

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

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

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

The President-Elect shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary-Treasurer for the Association. The Secretary-Treasurer shall receive such salary and allowance as may be fixed by the Executive Committee.
CONSTITUTION AND BY-LAWS

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

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

ARTICLE VI—PROGRAM COMMITTEE

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

ARTICLE VII—DUTIES OF OFFICERS

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

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

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

4. Second Vice-President: The second vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president, president-elect and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect and first vice-president. He shall be an ex-officio member of the Executive Committee.
CONSTITUTION AND BY-LAWS

5. Secretary-Treasurer. The Secretary-Treasurer shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee he shall publish said proceedings and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association. He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman of the Executive Committee a list giving the name, occupation and address of each applicant for individual membership for the approval of the Executive Committee. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, also an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars.

ARTICLE VIII—AMENDMENTS

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

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary-Treasurer.
President's Address
Reading of Papers.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers and eight members to Executive Committee.
Adjournment.

A suspension of the By-laws may be made by a two-thirds majority for the purpose of changing the order of business or to facilitate important business.

ARTICLE II—APPLICATIONS FOR MEMBERSHIP

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

ARTICLE III—MEETINGS

The annual meetings shall unless otherwise determined not less than thirty (30) days in advance by a majority of the members of the Executive Committee, be held at Chicago, Illinois, during the time of the International Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association. The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

ARTICLE IV—QUORUM

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

ARTICLE V—DUES

The dues for individual membership in this Association shall be five dollars ($5.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary-Treasurer of the Association. The dues for non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association. The dues for official memberships shall be one hundred dollars ($100.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
PROPOSED AMENDMENTS TO THE CONSTITUTION
AND BY-LAWS

T. J. Grennan and L. E. Bodenweiser

1. In ARTICLE III - MEMBERSHIP, amend lines 19 and 20 to read:

"There shall be five kinds of members—official, allied organization, individual, elected regional delegates, and non-voting juniors."

2. In ARTICLE III - MEMBERSHIP, between lines 26 and 27, insert the following:

"ALLIED ORGANIZATION MEMBERSHIP

Any non-profit 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."

3. In ARTICLE V - OFFICERS, under EXECUTIVE COMMITTEE, following the word "Association" on line 50, strike the remainder of the sentence and substitute therefor: ", not more than eight delegates at large representing the livestock industry, including poultry, and allied organization members."

4. In ARTICLE V - OFFICERS, under EXECUTIVE COMMITTEE, between lines 74 and 75, insert the following paragraph:

"The elected officers shall have the authority to place before the Executive Committee applications for allied organization membership. Not more than five such applications shall be presented to the Executive Committee for consideration at any annual meeting of the United States Livestock Sanitary Association."

5. In ARTICLE V - OFFICERS, under EXECUTIVE COMMITTEE, following the semicolon on line 88, strike the remainder of the paragraph, and substitute:

"and shall accept or reject applications for individual and for allied organization membership properly placed before them. Three negative votes shall disqualify for either such membership."
6. In ARTICLE VII - DUTIES OF OFFICERS, under paragraph 5. Secretary Treasurer, after the words "Executive Committee." on lines 144, insert the following sentence:

"He shall prepare forms for applicants for allied organization membership and shall notify each of the elected officers upon receipt of such completed application."

7. Amend ARTICLE VIII - AMENDMENTS to read:

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

PROPOSED AMENDMENTS TO THE BY-LAWS OF THE UNITED STATES LIVESTOCK SANITARY ASSOCIATION

1. In ARTICLE II - APPLICATIONS FOR MEMBERSHIP, between lines 180 and 181, add the following paragraph:

"Applications for allied organization membership shall be made in writing to the Secretary-Treasurer on an appropriate form prepared by him. In turn, notice of receipt of such application shall be provided each of the elected officers."

2. In ARTICLE II - APPLICATIONS FOR MEMBERSHIP, strike lines 181 and 182 and substitute the following:

"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."

3. In ARTICLE III - MEETINGS, strike lines 184 through 190, and substitute the following:

"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 livestock sanitary department of the state in which the meeting is to be held."

4. In ARTICLE IV - QUORUM, amend lines 200 and 201 to read:

"Thirty members of the Executive Committee shall constitute a
quorum, providing at least two-thirds of this number are executive of-
ficers representing the livestock sanitary departments of their respective
states."

5. In ARTICLE V - DUES, following the word "official" on line 209, insert
the words "and allied organization."

PROPOSED AMENDMENT RELATIVE TO THE
NAME OF THE ASSOCIATION

T. P. Siburt and R. A. Hendershott

I offer as an Amendment to the Constitution and By-Laws as follows:
that the name of the United States Livestock Sanitary Association be
changed to that of the American Animal Health Association.

T. P. Siburt, D.V.M.
Charleston, W. Virginia

R. A. Hendershott
Trenton, New Jersey
REPORT OF THE COMMITTEE ON RABIES

O. D. Dye, Atlanta, Georgia, Chairman; R. L. Burkhart, Washington Crossing, Pennsylvania; P. B. Dob, Springfield, Illinois; E. E. Saulmon, Hyattsville, Maryland; J. C. Shook, Harrisburg, Pennsylvania; L. E. Starr, Atlanta, Georgia

This report covers the calendar year of 1964. If this was a report of a commercial company selling a product, the picture would surely please the stockholders. Such is not the case. We are concerned with the incidence of rabies in the United States. Gentlemen, last year Doctor Kaley reported an increase of 206 cases in 1963 over 1962. This year, I am reporting an increase of 851 cases in 1964 over 1963. (In referring to Table I) Of great significance is the fact that seventy-five percent of the total cases reported were attributed to wildlife. Of the approximate 3500 cases of wildlife incidence reported, eighty-two percent were attributed to fox and skunk. (Again referring to Table I) Fox reports jumps from 622 cases in 1963 to 1,061 in 1964. Skunk reports jumps from 1,462 cases to 1,909 cases. Bat rabies reports were the highest in reporting service to date with a total of 352 cases reported from thirty-five states.

TABLE I

<table>
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<th>YEAR</th>
<th>DOGS</th>
<th>CATS</th>
<th>RATS</th>
<th>FOX</th>
<th>BATS</th>
<th>SKUNK</th>
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</tbody>
</table>

*86th U.S.A. Proceedings - 1964
Current Information - CDC
The canine and feline picture is quite gratifying. We can report a decline in canine rabies from a high 5,688 cases in 1953 to an all time low of 409 in 1964. With special emphasis on the canine facet of the rabies problem, eradication could be possible in the next ten years.
Therefore, the main conclusion that can be drawn from the data is that the increase of incidence of rabies in our wildlife population is reason for deep concern. (Please refer to the series of graphs 2, 3, 4 and 5). In Graph 2 Raccoon rabies has increased from approximately thirty-five cases in 1953 to 173 cases in 1964. 154 of these cases were reported in the Florida and South Georgia area. One puzzling aspect of this epidemic is

![Graph 2: Raccoon Rabies U.S.](#)

*68th USLSA Proceedings - 1964
Current Information - CDC

Graph 2
that the northward spread of this epidemic has stopped below the Altamaha River with no cases reported in any counties north of the river since the invasion from Florida was first reported in 1962.

Graph 3 shows that there has been a rather alarming increase of the incidence of bat rabies from eight cases in 1953 to 352 cases in 1964.

*68th USLSA Proceedings - 1964
Current Information - CDC

Graph 3
Graph 4 is quite revealing. From a high incidence of fox rabies in 1955-56 to a low in 1962, a new surge is reported in 1964 with a total of 1,061 cases compared to a low of 594 cases in 1962.

Of deepest concern to this assembly is the fact that skunk rabies has sky rocketed from a low of 319 cases in 1953 to 1,909 cases in 1964.

In order to give this assembly a broad look at the national rabies picture, I have prepared a map of the United States for the year 1964 showing

*68th USLSA Proceedings - 1964
Current Information - CDC
SKUNK RABIES U.S.

*68th USLSA Proceedings - 1964
Current Information - CDC

Graph 5
the incidence of rabies. This map will be published with this report in proceedings. Analyzing the reports spotted on this map of the usual wildlife species, and keeping in mind that eighty-two percent of the wildlife rabies was found in the report on fox and skunk, shows the concentration of skunk rabies incidence is in the Ohio Valley mainly north of the Ohio River and the upper midwest states of the Corn Belt. A total of 1,909 cases reported established a new high.

Appalachia seems to have the dubious honor of being home to the rabid fox. Reports also come from the lower Mississippi Valley for a national total of 1,061 cases.

Thirty-five states reported rabies in bats with the highest incidence, sixty-seven cases reported from Mississippi.

As mentioned previously, 154 cases of racoon rabies were reported from the Georgia-Florida area.

Always of high priority is the reporting of canine rabies. The brightest spot in the whole picture is the new low of 409 cases for 1964 with thirty-two percent of the cases reported from the Mexican border states, and twenty-six percent in the four contiguous states of the Mississippi-Ohio Valley.

I might report that the current status of the United States in 1965 has not shown any improvement. Rather, the first quarter of 1965 turned up
333 more confirmed reports than the same period of 1964, a twenty percent increase.

It is not with any idea of pride that I report just one human death from rabies in 1964—a decline from seven deaths in 1959. I wish I could report that we have had no deaths in 1964. A ten-year old boy from Minnesota was bitten by a rabid skunk and died twenty-six days later. With this in mind, may I reiterate a statement made by Doctor Kaley in his report last year, and I quote: "The fact that the reported rabies cases, both wildlife and cattle, represents only a small fraction of the actual number of cases, emphasized the serious wildlife, human health, and agricultural economic problem involved. We should face it realistically and energetically."

Therefore, the Committee on Rabies presents to this assembly the following recommendations:

1. The several states shall work toward the promulgation of Rules and Regulations to control the movement of caged, or otherwise restrained wildlife.

2. The Committee recognizes the outstanding work now being done and recommends additional emphasis be placed on studies in the field of epidemiology, vaccines and vaccination procedures, and laboratory procedures, and laboratory and field research in the wildlife rabies problem. More complete reporting on specific cases should be established for statistical evaluation.

It is further recommended that the United States Livestock Sanitary Association sponsor an outside Committee or advisory group with representation from state-federal wildlife agencies, state-federal public health services, and state-federal animal disease agencies to control and advise on the implementation of the above.

Gentlemen, I thank you.
REPORT OF THE COMMITTEE ON RESOLUTIONS

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

Chairman: This Committee received a Resolution from the Committee on Infectious Diseases of Cattle; while the Committee was in complete accord with the contents, and the merits, it felt it would receive much better attention and much faster action if it was turned over to the Committee on State and Federal Relations and asked that they make this a part of their program and budget request. That action was taken by the Committee.

The following Resolutions have been recommended by the Committee on Resolutions, and we ask your approval:

1. SALMONELLA

WHEREAS: The Committee on Transmissible Diseases of Poultry note with concern the increasing number of reports of Salmonella in humans. Public health officials state that many of these illnesses in humans are traceable to animal and poultry food sources. Several investigators report the contaminated animal and poultry feed, and the contaminated environment are a source of infection to animals, poultry and eventually to humans.

THEREFORE, BE IT RESOLVED that the President of the United States Livestock Sanitary Association consider the appointment of a Committee to be concerned with the scientific and professional aspects of the Salmonella problem.

Is there a motion to accept this Resolution? (Motioned) Is there a second? It's been moved and seconded that the Resolution be accepted. If there is no discussion, those in favor, vote by aye. Those opposed? Approved.

2. CATTLEGRUB CONTROL

WHEREAS: The use of organic phosphate compounds for the treatment and prevention of cattlegrubs have proved to be effective and economical, and

WHEREAS: Adverse effects and toxicity have been encountered under varying circumstances requiring further investigations which are now under way in an attempt to establish counter indications for the use of these compounds; therefore

BE IT RESOLVED that until more information from the toxicity
standpoint is developed from the practical use of these products, no broadscale cattlegrub control program involving these compounds be advocated at this time.

Is there a motion to accept this Resolution? Is there a second. It has been moved and seconded. If there is no objection, those in favor say aye; those opposed? The Resolution has been adopted.

3. TICK VECTORS

WHEREAS: Ticks play an important part of the transmission of anaplasmosis, and
WHEREAS: The present means for controlling these parasites are expensive and most difficult,

THEREFORE BE IT RESOLVED that this Association urge more research be conducted to develop better or more practical means of controlling and eliminating these vectors.

Is there a motion? I move its adoption. It has been moved and seconded with no opposition that the Resolution be adopted. Those in favor, vote by aye. Those opposed? So adopted.

4. SHEEP SCABIES

WHEREAS: Within the various States there have been most commendable progress in the eradication of sheep scabies, and
WHEREAS: This disease has been reduced to its lowest level in our history of scabies,

THEREFORE BE IT RESOLVED that this Association and all Regulatory Officials exert every effort this winter to conduct an eradication program which would complete eradication of psoroptic sheep scabies.

Is there a motion to adopt? And a second? Seconded. Those in favor, signify vote by saying aye. Those opposed? So adopted.

5. IMPORT REGULATIONS

WHEREAS: The importation into the United States of animals and birds constitute a hazard insofar as disease and parasite introduction is concerned, and
WHEREAS: The existing Federal Import Regulations are applicable only to ruminants, swine, equines, poultry and certain dogs—

THEREFORE, BE IT RESOLVED that Federal Import Health Regulations now applicable to livestock and poultry, be made applicable to all birds and all mammals except man, insofar as such regulations pertain to diseases and parasites that might be introduced into the United States.

Is there a motion to adopt? The motion has been made to adopt. Is there a second? Those in favor, signify by aye. Those opposed? The Resolution has been adopted.
6. RESOLUTION ON CHANGE OF THE NAME OF THIS ASSOCIATION SUBMITTED BY THE WESTERN STATES LIVESTOCK SANITARY ASSOCIATION

WHEREAS: The present name of the United States Livestock Sanitary Association does not completely reflect the scope of activities of this organization, and

WHEREAS: The words "livestock" and "sanitary" as used in the name are frequently misunderstood by the general public and other professional groups and hence deemed inappropriate—

NOW, THEREFORE, BE IT RESOLVED that the Executive Committee give consideration to changing the name of this organization to one which more fully defines the activities of regulatory medicine and associated industry groups.

Is there a motion to adopt? Is there a motion to second? The motion is made and seconded. Those in favor, signify by "aye." Those opposed? The Resolution has been adopted.

The Committee requests that the Secretary prepare the usual housekeeping resolutions.

Mr. Chairman, this concludes the Report of the Committee on Resolutions.
REPORT OF THE COMMITTEE ON DISEASES OF SHEEP AND GOATS

L. E. Bodenweiser, Albuquerque, New Mexico, Chairman; P. C. Bennett, Ames, Iowa; A. A. Erdmann, Madison, Wisconsin; W. J. Hadlow, Hamilton, Montana; J. L. Hourrigan, Hyattsville, Maryland; A. K. Kuttler, Boise, Idaho; H. Marsh, Bozeman, Montana; B. McGowan, Davis, California; M. D. Mitchell, Pierre, South Dakota; W. T. Oglesby, Baton Rouge, Louisiana; R. I. Port, Cheyenne, Wyoming; O. H. Timm, Dixon, California; W. Van Horn, Buffalo, South Dakota; H. Versluis, Salt Lake City, Utah; S. B. Walker, Austin, Texas

DR. L. E. BODENWEISER: Dr. Safford, Members of the United States Livestock Sanitary Committee; I wish to discuss the Report of the Committee on Diseases of Sheep and Goats. The Committee's Report this year embraces three diseases: Footrot, Epididimitis, and Scrapie.

FOOTROT

The Committee recommends the adoption by all States of a program which will provide for the following: (1) Sheep infected with Footrot will be placed under quarantine by the Office of the State Veterinarian. Movement of animals from quarantined flocks will be allowed under the following conditions: (a) by permit of the State Veterinarian's Office for immediate slaughter; (b) by permit from the State Veterinarian's Office, weened lambs may be moved for further feeding, provided they are put through an approved footbath at the time they are moved from the quarantined premises. Lambs found to be infected must stay on the quarantined premises or go directly to slaughter. (2) Under supervision of the State Veterinarian's Office, infected flocks will be treated as soon as possible after a diagnosis is made. Treatment will follow the approved procedures for treatment of flocks infected with footrot as outlined in Farmers Bulletin 2206 United States Department of Agriculture. This Bulletin was prepared with the assistance of Doctors Hadley Marsh, former Chairman of the National Committee on Footrot in Sheep, and Doctor Blaine McGowan, present Chairman, National Committee on footrot in sheep. (3) The State Veterinarian's Office will terminate quarantine on the basis of two clean inspections: (a) the first clean inspection will be at least thirty days after the last infection has been found and eliminated from the flock; the second clean inspection will be made at least thirty days after the first one. (4) All sheep entering a sales yard will be visually inspected upon arrival. The diagnosis of footrot in one animal will place the whole group or lot on an infected-flock basis and will be handled as follows: (a) Immediately after diagnosis, the infected flock will be placed in isolated pens, and will not be co-mingled with any sheep other than those previously or subsequently diagnosed as infected with footrot. (b) Infected
flocks will be placed under immediate quarantine by the State Veterinarian's Office, and will be moved from the sales yard within 48 hours after quarantine is imposed, in one of the two following manners: 1; By permit for immediate slaughter. 2. By permit, and still under quarantine to owner's premises, following which it will be handled as described under Regulations 1, 2 and 3 above. 3. Pens and sales yards used to isolate sheep infected with footrot may be used repeatedly and continuously for this purpose; however, no sheep free of footrot nor any other animal species may be placed in these pens before the 15th day after removal from these pens of the last footrot infected sheep. Pens, alleys, and chutes used by infected sheep shall be cleaned and disinfected immediately if they are to be used by noninfected sheep within a 15-day period. 4. All vehicles in which footrot infected sheep have been transported will be cleaned and disinfected under supervision of the State Veterinarian's Office, or Federal Inspector in charge, before sheep or any other species of animals are to be transported in said vehicles. 5. Health Certificates for Interstate Movement of Sheep other than for immediate slaughter, will henceforth specifically require that the sheep covered by the certificate have been inspected and were found free of footrot.

EPIDIDYMISIS

It is recognized that Ram Epididymitis is gradually spreading to, and being diagnosed in an additional number of States every year. Movement of infection from Western States to the Midwest and East is slow and insidious, but definite. Diagnosis of this disease may be accomplished only by careful examination of rams by experienced veterinarians plus appropriate serological testing. States in which this disease has not yet been recognized are strongly urged to actively determine whether or not ram epididymitis actually exists within their boundaries. States which to date enjoy a low incidence of this disease are urged to consider control measures employing physical examination, blood testing and vaccination of clean rams with a view to limiting further spread, or eradicating this disease. Furthermore, all States are strongly urged to consider regulations which would insure the importation for breeding purposes of only clean, properly vaccinated rams.

SCRAPIE

During the year there were significant changes in the procedures followed in the scrapie eradication program. These changes came about as a result of the research information presented at the Scrapie Seminar held in January 1964, and also due to experience with the disease in the various States of this country as well as in Canada. During the last meeting of your Committee in Memphis, Tennessee, October 1964, we discussed proposed changes and revised and recommended modifications in the program. The General Assembly of the United States Livestock Sanitary Association approved the Committee's recommendations and a
modified program was placed in effect. During the fiscal year 1965, there were 12 outbreaks of Scrapie in seven States, bringing the total since 1947 to 150 outbreaks in 27 states. The 12 outbreaks in 1965 included the following: California—two (in Los Angeles and Sacramento counties); Illinois—two (in Champaign and Hancock counties); New York—one (in Tomkins County); Texas—two (in Sutton and Hill Counties); Missouri—two (in Clinton and Lynn counties); Virginia—two (in August and Gutzland counties); and Kansas—one (in Caola county).

All sheep found to have Scrapie were of the Suffolk breed, with the exception of an infected ewe in a Champaign County, Illinois flock which was a Hampshire. Of the 246 affected sheep found in the United States, one was a Hampshire, 12 were Cheviots, and the remaining 233 were Suffolks. During the year, 2,097 sheep were slaughtered under the program, a considerable decrease from the previous year when 15,192 were slaughtered. Part of this reduction is attributable to the modifications of the program. The fact that the infected flocks were smaller than some found the previous year also aided in reduction. For some time, members of the sheep industry have advocated more scrapie research efforts, both in this country and abroad, including the holding of sheep from an infected flock to determine how many of these animals would develop scrapie if held for extended observation rather than being slaughtered. The Texas Sheep and Goat Raisers Association, National Woolgrowers Association, Committee on Diseases of Sheep and Goats, of this Association and others, have been in favor of such studies.

SCREWWORMS

The Southwestern Screwworm Eradication Program requires a large facility for sterile fly production. The site chosen was Moore Air Base, Mission, Texas. This facility was acquired by the United States Department of Agriculture, and included a substantial acreage of native grass, suitable for holding test sheep and goats on pasture for an extended period of time.

(This is not in the report, but I would like to add at this time that this is all under security fence).

The study is in the nature of a field trial to learn by observation what happens to sheep and goats raised on contaminated premises. It is planned that only natural scrapie will be involved in the field study. Animals in the field trial will include both close relatives, blood-line sheep of known affected animals and other animals both goats and sheep which are not related to, and have never been exposed to, scrapie affected animals. The scrapie free sheep will include 30 ewes and three rams of four breeds: Rambouillet, Targee, Hampshire and Suffolk; and will be pastured with the blood line relatives of scrapie affected sheep. The goats will include one male and ten female angoras; five female Toggenburgs; five female Nubians; and one male goat of either breed. Understandably, the sheep taken to Mission so far have largely been blood-line sheep identified as outbreaks occur. Sheep in this category include sire or dame of affected
sheep, and get progeny of sire or affected dame full and half-sibs of affected sheep, grand progeny of affected sheep and grand progeny of affected parents of affected sheep. Presently, this includes some 122 head. Of the sheep taken to Mission, either for observation or for the field trials, nine have died of scrapie.

The purchase of scrapie-free sheep is moving along more slowly. So far, 33 Rambouillet, 33 Targees, 11 Hampshire, and some of the goats have been purchased. Efforts are under way to locate the remaining test sheep. In order that the results of the field trial be meaningful, the scrapie-free test sheep must meet strict criteria from both a contact and bloodline standpoint.

Mouse Inoculations as an Adjunct to Histopathological Studies:

Arrangements have been made at the National Animal Disease Laboratory, Ames, Iowa, to inoculate mice with tissues from scrapie-suspect sheep. This should be particularly helpful in those cases which are highly suspicious from a clinical standpoint, but from which tissues suitable for histopathological studies are not available. Of the tissue samples from histopathological positive sheep furnished the research workers at the National Institutes of Health, Department of Health, Education and Welfare, eight have been positive on mouse inoculation including tissues from both Chevoit and Suffolk sheep.
The Committee on Program and Policy met upon three occasions this year. The first of these meetings was held in Washington, D.C., during the latter part of March at which First Vice-President Grant Kaley served as chairman in the absence of the permanent chairman. As is customary in the first of the two Washington meetings, the Committee had the opportunity to review with the United States Department of Agriculture scientists their budgetary proposals for the forthcoming year (in this case, Fiscal Year 1966), and the following statement was prepared for presentation to the Congress as a result of these deliberations:

"This Association has been coming to Congress for several years, and more especially to these subcommittees, to discuss the Department of Agriculture, Agricultural Research Service, appropriation and to make requests, which in the opinion of our Association are in the public interests and necessary to continue the orderly and effective control and eradication of contagious and infectious diseases of the nation's livestock and poultry. For this year it has been decided to submit a written report in lieu of a personal appearance before these committees and we herewith submit our recommendations.

"For the opportunity of presenting these views before the Subcommittee we are most grateful. You will note that in the Budget Estimates for 1966 there are several items that show an increase over 1965. We will emphasize those items that we deem most important. A summary of these proposals follows:

**Animal Disease and Pest Control**

**Animal Disease Control and Eradication**

**Eradicating Brucellosis**

"We hope that Congress will continue this item at the existing rate which is proposed at $23,958,300. A few years ago it was asked that Federal appropriations be increased to $20,000,000 to complete the job of brucellosis eradication. At that time those petitioning for this hoped that by 1965 the entire nation would be modified certified and that we would then discuss the cost and time of complete eradication. We will not quite meet this time table and currently estimate that the entire nation will be modified certified at the end of 1967. On the positive side we must point out, circumstances were such that we have achieved total eradication in
some states where it was not contemplated; these achievements counterbalance some of the deficiencies. Insofar as the total program is concerned, we are equal or ahead of schedule. Under existing financing (Federal and State) we expect to be able to discuss fund reduction beginning with fiscal 1968 as it relates to brucellosis.

**Eradicating Screwworms**

"The United States Livestock Sanitary Association is very pleased with the progress in Screwworm Biological Control, and the progress attained in attacking this animal and human pest is very commendable. We are concerned, however, that the possibilities of re-infestation from Mexico presents a potential hazard. We feel the logical point of surveillance would be at Mexico's narrowest point. This, of course, would involve freeing all of the portion of Mexico north of that line, and we do realize there are other factors involved that do not directly involve screwworm eradication. We feel, however, that the Congress will in the final analysis do justice to this cause.

**Eradicating Hog Cholera**

"We support the $4,615,500 for this program. By the end of fiscal 1966 all states will be beyond phase I or preparatory state of the program, and will be in the active eradication phase which will require a $1,000,000 increase in appropriation. This program so far has reduced the incidence of Hog Cholera from 2,040 outbreaks in the first quarter of 1961, to 450 outbreaks in the last quarter of 1964. This is a disease which we feel with adequate financing and intensive control, total eradication can be accomplished in a few years. Then there will be a reduction in the cost of this program to a lower figure which will be needed for surveillance purposes.

**Animal Inspection and Quarantine**

**Import-Export Inspection and Quarantine**

"We appreciate the support given to this item by the Committee to previous requests by the United States Livestock Sanitary Association. We have been asking Congress for additional people and facilities at air, ocean and land ports of entry for livestock and livestock products. With an increase in air travel it is a constant and expanding problem for those charged with the obligation of keeping out foreign plagues. We are pleased to see the Administration has proposed another $100,000 for this area and sincerely hope it will be supported by your Congressional committees. Congress increased this appropriation by $100,000 in 1963, by $86,000 in 1964, and $185,000 in 1965. This has provided increased inspection and greater security at the ports of Boston, Philadelphia, New York, Norfolk, Charleston, Miami, Honolulu, San Juan, New Orleans, Corpus Christi, Port Arthur, Los Angeles, San Francisco and Portland, Oregon. There is a request for an additional $100,000 in the 1966 budget which would provide
REPORT OF COMMITTEE

for increased inspection at Buffalo, San Juan, Chicago, Houston, Portland, Oregon, and Norfolk. Much of this money is used for additional personnel, since it takes many people to screen the enormous amount of material, living and dead, which comes into this country. We appreciate your past support and ask for your continued support with respect to facilities which have been greatly improved. We are pleased that the Clifton, New Jersey, facility is being sold since it is inadequate for the New York City port. The sale from this facility should bring around $350,000 and in the near future additional budget requests will be made to replace this facility. We are pleased that the Department of Agriculture is in the process of working out with the Dade County, Florida, Port Authority, details for a quarantine facility to meet United States Department of Agriculture standards at the approximate cost of $560,000. Details for a 20-year lease are being worked out, at the end of which, ownership will revert to the Department of Agriculture. It is gratifying that Congress has seen fit to construct inspection and quarantine stations along the Canadian border where there is much traffic in livestock. Those at Holton, Maine, and Blaine, Washington, have been completed. The Canadian government has also built some stations, and we have an excellent agreement for shared use which eliminates duplication and demonstrates the kind of cooperation, of which we are so proud. We are also pleased to note that in the absence of Federal money, the Government has negotiated for private construction of a station at Portal, North Dakota, which will be leased.

Control of Manufacture, Importation, Shipment and Marketing of Viruses, Serums, Toxins, etc.

"In the control of Veterinary Biologics the United States Livestock Sanitary Association has been asking for increases for several years, and are pleased to note that Congress has responded generously. However, an adequate service is still not being provided in this field. The facilities we now have are being used to a maximum. We note with pleasure that an additional $50,000 is provided in the proposed budget and sincerely hope the Committee will support this sum. We report the use of the increase of $750,000 over a three year period for the following purposes: In 1962, of the rabies vaccine tested, 46 percent of the lots were found to be ineffective. Under the new procedures in 1964, only 1.5 percent of the lots tested were found to be ineffective. There are biological products on the market for 50 known diseases of livestock and poultry. In excess of 4.5 billion doses are marketed annually. In the last 15 years modern technology and newer knowledge, particularly of viruses, has resulted in a considerable change in biological products used for the prevention of disease. Fifteen years ago 90 percent were killed products or bacterins. Today 90 percent contain living organisms and are potentially dangerous. Before Congress realized the danger and began surveillance of these products, the only ones checked were those in use by the United States Department of Agriculture programs, and surveillance of others was inadequate. Funds were increased for this work, at the request of various agencies, and conditions
have improved; however, as of now surveillance is only given to products used in the control of about twenty diseases. Of this twenty, in 1962, 17 percent of the lots produced were checked for effectiveness, eight percent of which were found unsatisfactory. In 1963 an acceleration in surveillance was begun. Twenty-six percent of the lots of biologics intended for use in 20 pertinent diseases were checked, and 13 percent of these products were found to be unsatisfactory. In 1964, with the increased assistance furnished by Congress, 38 percent of all lots of biological products used for these aforementioned 20 diseases were checked and 6 percent were found to be unsatisfactory; a marked improvement over 1963. Some of these 20 diseases affect animals and man. To do the job correctly and to provide adequate coverage, we feel it is going to take about 5 million tests a year, and we are only reaching about 50 percent of this goal now. We are not asking for more money at present, since most of the facilities are being used to the maximum. The need exists now, however, for about twice as much laboratory capacity. Requests for this will be forthcoming in the near future. We must bear in mind that surveillance is a continuing proposition.

PESTICIDES REGULATION

"We are advised as a result of urging that the United States Department of Agriculture has taken a more aggressive position with regard to toxic substances in feed and food supplies. We are pleased to see the Congress recognizing this as evidenced by an increase in the 1964 figure of $1,479,600 to $2,664,900 in 1965, and a proposed increase to $3,714,900 for the fiscal year 1966. It is understood that the Congress is considering the construction of a new facility at College Station, Texas, for a toxicology research laboratory with an estimated cost of $3,000,000. No funds are being requested in the 1966 budget for this, but we hope the Committee will look with favor on the request of an appropriation for engineering and planning of this facility.

RESEARCH

Farm Research

"We would like to call to the Committee's attention that information on total research facilities and personnel is still lacking. This has been discussed previously with the Committee and we would like to know whether or not a report or a study has been made. At this time we will not make recommendations that might be of a duplicating nature. It has previously been recommended and urged to the Department and to Congress that the Beltsville facility be converted to a facility for parasite research. The estimated cost of conversion has increased from 3.5 to 6.5 million dollars. We urge the Committee to find out why this previous recommendation, which was approved, has not been acted upon.

"We would like to thank the committees for the opportunity of making these presentations; the United States Livestock Sanitary Association is
ready and willing to be at the disposal of the Congress at any time."

The second of the Washington meetings took place on May 24, 25, and 26, so as to consider and assist in the formulation of the 1967 Fiscal Year budgets of the U. S. Department of Agriculture as they relate to animal disease control and eradication affecting the several states. Your Committee wishes to make this observation with respect to this extended two and a half day's session: While the additional time allotted this particular meeting for reviewing proposals and in preparation of our report to the Agricultural Research Service Budget Review Committee did preclude burning some midnight oil, as has been the custom for several years, it is felt that following the Division's presentations at least a full day should be allotted to our Committee for drafting the comprehensive recommendations directed to the Department's Review Board. It is a very difficult task to prepare a complete and logical evaluation of the Department's animal disease programs in an extended night session which follows an entire day's deliberations on these subjects. We hope that the Agricultural Research Service officials will take this into consideration prior to scheduling next year's meetings in which we appreciate participating.

Our Committee recommendations to the Consumer and Marketing Service and Agricultural Research Service Review Boards on the 1967 Fiscal Year budgets were as follows:

MEAT INSPECTION

"The Committee has reviewed the meat inspection and poultry inspection programs, the former having recently been assigned to the Consumer and Marketing Service from the Agricultural Research Service, and wish to recommend the following:

1. Sufficient funds should be made available to keep abreast of the increasing public demand for meat inspection. Historically we are behind in providing sufficient funds to cover this mandatory service.

2. It is our view that funds to pay for this service should continue to be derived out of appropriations from the general tax revenues. We continue firm in our belief that this renowned service be free from any possible conflict of interest. Human nature being what it is, it is inconceivable to us that all packers could be above temptation.

3. Recent disclosures of the inclusion of tissue from dead and moribund animals in products for human consumption points to the need for closer state-federal cooperation in maintaining continued surveillance on the disposition of diseased and unwholesome meats to prevent them from entering trade channels.

4. The proposed bill ('Compendium of Ideas') to clarify and otherwise amend the meat inspection act was received by us immediately prior to this session. Upon cursory examination it is evident that there is some language that is objectionable to some of the states; however, it bears further study and we will, within 60 days, present a supplemental statement on this proposal should we find it warranted.
5. The exemptions from federal meat inspection authorized by Title I, we feel, should be reviewed with the thought of eliminating all of the provisions that present a threat to the effective enforcement of the intent of the act. We are confident that the public, as well as we here making this presentation, cannot but look with jaundiced eye on the exemptions which permit individuals to circumvent the public health protection in the production, interstate movement, and sale of uninspected meat to individual consumers and restaurants.

ANIMAL INSPECTION AND QUARANTINE

"The threat of invasion by foreign animal pathogens continues to be a major concern in the absence of inspection service to permit continuous surveillance of meats and meat products originating abroad and control of garbage containing such meat and meat products.

"In the last years some improvement has been noted in the inspection service at some dozen ports of entry. Others still appear to remain understaffed. There is an indicated need for sufficient inspectors to seal meat lockers on all vessels arriving from foreign ports and to insure the safe disposition of galley scraps.

"An additional need is for complete inspection of baggage landed at inland airports following direct flights from abroad. Additional surveillance is needed on the handling and ultimate disposition of materials brought into the continental United States from abroad for research purposes as well as the ever increasing quantities of animal products and by-products such as bone, bone meal, hides, etc.

TESTING OF BIOLOGICS

"We are pleased to note the degree of testing of biological products that has been accomplished to date with the existing facilities. We are also pleased with the improvements in the product quality as a result of the close checking of given products. However, during the past two years the testing surveillance has covered less than half of the products licensed and less than half of the diseases for which biologics are used. Testing, therefore, has of necessity been on a product priority basis. Consideration should be given to adequate increases for additional testing of biologics and for the construction or lease of additional testing facilities. Our ultimate goal is that every veterinary biologic should be subjected to such tests as may be necessary to insure potency, safety and purity. In the case of some products, effective tests have yet to be developed. Even though these testing procedures may cost an average of $20,000 to develop, it is urged that funds be made available to complete this work as rapidly as possible. Once satisfactory methods of determining safety and potency have been developed this work need not be repeated. The livestock industry of the United States is currently depending immunologically upon some 50 products for which adequate checks and safeguards are not presently available. The need for further attention to this area is
emphasized by the number of products which have been tested and found to be unsatisfactory.

"Failure to check biologics may result in marketing of a product which may be defective in one of four ways.

1. Insufficiently antigenic.
2. Dangerously potent.
3. Contain substances which may induce state of anaphylaxis (example—rabbit serum in lepto biologics).
4. Contain pathogens other than the one against which immunity is desired.

ANIMAL DISEASE AND PARASITE

"The needs for research in areas of animal disease are increasing constantly with new problems developing from changing livestock environment and rapid transportation. The attrition resulting from general wage and salary increases from yearly budgets has caused a net loss in research expenditures. This sum is in the area of $1,000,000 in the past six years. This Committee feels that annual salary increases should be supplemented without decreasing the research budget.

"Continuously over the past five years we have requested an appropriation to convert the buildings and facilities at Beltsville into a parasitology research center. Our original request for this conversion some five years ago was $3,500,000. Through delays in providing this building at that time, this same request today will cost double that. This is an area that has long been neglected and is an important item in animal production.

"In the 1967 budget, this Committee recommends consideration be given to the following areas:

1. That funds be allocated to proceed with equipping and staffing the veterinary toxicology insect research laboratory. We hope that this goes forth as soon as possible so that actual operation can commence during the 1967 fiscal year and there will be no delay in opening for business when the facility is available.
2. The problem of swine abscesses is currently demanding research. We recommend an allocation of $250,000 for the study of this problem.
3. Increased anaplasmosis research is demanded and justified by the livestock industry. We recommend an increase of $500,000 in this project.
4. For the protection of the growing 1-1/2 billion dollar horse industry, an allocation of $200,000 is recommended for increased research in equine piroplasmosis.
5. The last report of the Committee on Vesicular Diseases of the United States Livestock Sanitary Association recommend investigation regarding the transmission and reservoirs of vesicular stomatitis virus. The 17 western states at the last annual meeting of this regional association, are on record concurring in this request. We do not believe that a
research project of the scope of foot and mouth virus investigation is necessary to provide the needed knowledge in this area for protection from other forms of vesicular diseases, but that additional knowledge in regard to VS is necessary.

"The following non-recurring items for construction are recommended:

1. We recommend a $6,500,000 budget item for conversion of the Beltsville facility, adapting it for parasite research, one of the major needs in veterinary medicine at this time. The more concentrated agricultural activities and lack of new land is greatly multiplying the parasite problem the livestock industry is facing.

2. There is presently a need for $2,500,000 for expanding research and construction of an additional animal wing at the Plum Island facility. We are on the threshold of a possible breakthrough in the area of immuno-chemistry. This could lead to the world's first developed pure vaccine with subsequent synthetic production of pure antigens for many diseases.

3. Needs are present for additional studies of bovine leukosis and other neoplastic diseases of livestock. Funds should be considered in this area for a facility and investigation of this entity.

4. We recommend allocation of $299,000 for construction of insect proof houses, additional incinerator facilities, and other additions to the Denver laboratory, to further the investigation of blue tongue virus. We feel this work is urgent in that the common market in countries where blue tongue is non-existant could well impose an embargo on United States beef carrying this virus.

5. The sum of $750,000 is recommended for increasing facilities at the Logan, Utah, plant toxicology laboratory.

BRUCELLOSIS

"We would like to point out that the states are presently contributing to the brucellosis program beyond the 60-40 basis, and in fact, more than 50-50. If federal funds are continued at the same rate through fiscal 1967, it is anticipated that the entire country will be a modified certified brucellosis area at that time. At the same time, we should have reached a goal, at the rate of present financing, of having approximately 1,000 counties in certified brucellosis free status.

TUBERCULOSIS

"Contributions made by the states in the tuberculosis program is approximately eight dollars state to three dollars federal. An increase of $1,210,000 for T.B. should provide additional emphasis for identification of livestock in tracing of infected animals, as well as development of an identification system that would apply in the eradication of tuberculosis from swine and poultry. It should also provide for additional personnel for accelerated epidemiological studies.
"It is our understanding that the work of Dr. W. L. Mallman on mycobacteria research will end as of December 31, 1965. It is strongly recommended that funds be provided to continue this work after January 1, 1966.

MISCELLANEOUS DISEASES

"Under the miscellaneous diseases category, some $800,000 has been requested of Congress for the 1966 fiscal year. This amount is contemplated to be used on such diseases as those affecting poultry, scrapie, blue tongue, equine piroplasmosis, vesicular diseases, diseases of an exotic nature, and possibly others. This committee is of the opinion that in line with previous requests those diseases affecting poultry should be set up as a line item.

"We feel that the same separation should be made for equine piroplasmosis, which is assuming greater economic significance with relation to the horse industry of the country.

"For the inauguration of studies on a disease which is greatly affecting the economy of the dairy industry of this country, it is felt that $100,000 should be included for initiating pilot studies on mastitis for participation in state programs. We understand that the National Mastitis Council shares this feeling.

"We have been advised that several states have voiced concern with an entity that is becoming more of a problem in their particular areas, this being bovine leukemia. Research people need epidemiology associated with the incidence of this disease, and it is recommended that $100,000 be made available for cooperation with university studies in several states.

POULTRY DISEASES

"We are concerned with the ever broadening problems of the poultry industry and the many segments of our society that are becoming involved. The commercial poultry industry has vital disease problems. They are deeply involved in this matter of salmonella infections which are of concern to us both as livestock sanitarians and consumers. The feed industry is concerned with their part in this overall salmonella problem in which they have a continuing responsibility. The principle cause of condemnations in the slaughter house, both of chickens and turkeys, is this respiratory complex that we now rightly or wrongly characterize as mycoplasmosis. CRD in chickens, sinusitis in turkeys are still involved in this mycoplasma syndrome. Requests have been made for funds to establish a pilot study for eradication of two important poultry diseases that are salmonella caused in one section of the nation. Requests have been made for an appreciable sum of money by the feed industry to study this problem and to solve it. We are concerned with the mycoplasma problem which is not directly related to salmonellas. Our point is that it is time and past time, which we have called to this Department's attention
before, that the whole area of poultry diseases be taken out of the miscellaneous category and set up as a specific budgetary item by itself so that all aspects of this industry can receive the attention that is long overdue.

**EQUINE PIROPLASmosis**

"Somewhat over three years ago there was recognized in the United States for the first time a disease which has since become of great concern to the horse industry of the country. Many of the answers to the problems concerned with equine piroplasmosis had to be developed by this Department, cooperating with the State of Florida. During this relatively short period of time, we have made remarkable progress in obtaining the answers to many of these questions, but subsequent complications have clouded the picture and necessitates further investigations. It is therefore recommended that in view of its far reaching implications to this multi-billion dollar industry that the appropriation to continue work on this disease be augmented by some $65,000 and set up as a separate item in the forthcoming budget.

**Hog cholera**

"It is very encouraging to note the rapidity with which the hog cholera eradication program is moving towards its eventual goal, and with the increased activity into the final phases, particularly by some of the more populous swine producing states of the Mid-West, it is apparent that if the program is to reach a successful conclusion, additional indemnity monies must be made available for participation with these states, and to see the program through to its final conclusion.

"As the states have advanced into the final phases of the program, the condemnation rates in slaughter houses has decreased to a point where it is quite appreciable as a direct result of a concentrated hog cholera eradication program.

"At this crucial period it is essential that sufficient indemnity funds be provided to stamp out the last vestige of the disease, and consideration must be given to providing the necessary indemnity to effectuate this.

**INTERSTATE INSPECTION**

"An increase of $290,000 is needed in the area of interstate inspection:

1. To study patterns of livestock movement.
2. Special projects in animal identification.
3. Increased training of personnel in the health problems of livestock in transit.
4. To expand inspection of animals in markets."

The third meeting of the Committee was held in Portland, Oregon, on July 13, 1965. We were apprised of the latest developments in the U.S.
Department of Agriculture's budgets and appropriations by Dr. R. J. Anderson, after which the matter of the so-called "Compendium of Ideas" was fully discussed again as it relates to extension of Meat Inspection and other matters. We had the benefit of having Drs. Somers and Pals of Consumer and Marketing Services present for a full and free discussion of the subject.

Following this discussion in executive session, the Committee unanimously agreed that the United States Department of Agriculture should not carry this whole matter further until basic agreement had been reached on the principal points in controversy. A resolution to this effect was passed without opposition, which placed the United States Livestock Sanitary Association through this Committee in concurrence with the position heretofore taken by regional affiliates of the National Association of State Departments of Agriculture.

This also endorsed the United States Department of Agriculture's stated position as specifically made to our Committee in Washington, D. C., that they desired that all involved groups be in agreement regarding the provisions of the compendium before presenting it to the Congress as an administrative bill.

It is understood, however, that in spite of the fact that the involved groups are still not in agreement regarding its provisions, a bill incorporating most of the points as contained in the compendium was last week presented to the Congress. It is presumed that no Congressional action will be taken on it during this session which will afford the Department an opportunity to attempt to resolve with industry the bill's controversial issues prior to its consideration before the next session of Congress.

Your Committee wishes to direct the Association's attention to its action at San Francisco in 1959 when the Committee on Legislation and the Advisory Committee to the Agricultural Research Service were combined to become the Committee on Federal Programs and Policy. Since that time, through usage and ease of identification, it has come to be known as the Committee on Program and Policy. The function of the Committee, however, deals in the main with state-federal relations and in an advisory capacity toward the formulation of federal budgets. In view of this, your Committee recommends that without altering its composition or function the Committee name be changed so that hereafter it be designated the "Committee on State-Federal Relations."

I would like to state that my job as Chairman has been much more easily and enjoyably accomplished this year through the sincere efforts of all of the individual hardworking members of the Committee. I wish also to express personal appreciation to my assistant, Wilson Powell, who, although not a member of the Committee, has lent invaluable aid to me as to other members in the preparation of reports of the Committee.
REPORT OF THE COMMITTEE ON STOCKYARDS,
MARKETS AND TRANSPORTATION

D. A. McGill, Olympia, Washington, Chairman; W. E. Bechdolt, South St.
Paul, Minnesota; J. W. Black, Hinsdale, Montana; M. C. Mitchell, Pierre, South Dakota; A. G. Pickett, Topeka, Kansas; C. T. Sanders, Kansas City, Missouri; Jay B. Smith, Columbus, Ohio; W. M. Thompson, Phoenix, Arizona; F. W. Hansen, Jr., Hyattsville, Maryland

The Committee reviewed the report of last year's meeting relative to recommendations regarding the federal form #48 and asked for a report from committee members who had been working on its revision. Drs. Hansen and Erickson reported some progress in modification of the form and possible replacement with a uniform health certificate. It was the consensus of the Committee that the form #48 should reflect sufficient information that it could be accepted by the receiving state in lieu of an Official Health Certificate. The Committee complimented the Stockyards Division of ANH for their efforts and requested that they continue along these lines to bring this matter to a conclusion as soon as possible. The Committee feels that the value of the form used for release and health certification on animals leaving the market is directly related to the market operator's acceptance of his responsibility to allow no animals to be released from the market until proper health papers are obtained.

The matter of identification of livestock in movements was discussed at length in light of recommendations made by various committees of the United States Livestock Sanitary Association. The Committee moved to support the feasibility study recommended by the Committee on Rules and Regulations relative to livestock identification. The Committee respectfully requests representation on such a study committee in order to assure that any recommended identification procedure does not place undue hardship or restriction on the orderly movement of livestock through normal marketing channels.

Members of the Committee in attendance at meetings last summer with representatives of industry, Federal Stockyards and Certified Public Livestock Markets in sessions at Washington D. C. and Kansas City, Missouri reported convictions of honest concern on the part of representatives present and a mutuality of understanding of their respective responsibilities in assuring maximum inspection of livestock and adherence to health standards, with minimum interference with market movements. The Committee expressed the hope that such conferences be continued to better achieve mutual understanding of problems.

The compiled results of the questionnaire survey conducted by the Committee and studied by the individual members previous to this meeting was reviewed by the Committee in light of information gained; with particular emphasis placed on the comments on "Specifically approved public livestock markets." The Committee then discussed again the
necessity and desired status of markets specifically approved under Part 78 and 76 title 9 C.F.R. The Committee concluded that there is still a definite need of specifically approved markets in many areas to facilitate the orderly movement of livestock to market. The Committee also recognized the area of necessity for specific approval of markets in close competition with Federal Stockyards. The Committee recognized too that much confusion exists among shippers as to the purpose and privileges relative to specific approval. The Committee therefore requests the Stockyard Division of ANH to further study the feasibility of providing specific approval contracts on the basis of qualifying a market to handle all classes of livestock under the same privileges and responsibilities afforded a federal stockyard, except that ANH would not be responsible for providing the inspection services in such a market. The Committee recounted the several reports received that the specifically approved market system is being abused in some areas and is thus contributing to diversion of some shipments of livestock. The Committee therefore recommends that the United States Livestock Sanitary Association request State and Federal Livestock Officials to immediately undertake a review of their states specifically approved markets and work with industry to put a stop to such evasions and abuses by giving credence to removal of Specifically Approved status from markets no longer showing a need for such status.

Others in attendance at the meeting in Lansing were: R. E. Sneddon, Secretary of American National Cattlemens Association, Denver, Colorado; J. W. Prince, Certified Market Operator, Elkinsin, Michigan; J. W. Trumble, Assistant State Veterinarian, Lansing Michigan; Dean E. Flagg, State Veterinarian, Bismarck, North Dakota; I. Erickson, ANH, Hyattsville, Maryland; Charles McClintic, W. Virginia Department of Agriculture, Beverly; Ingvard Svarre, Certified Market Operator, Sidney, Montana.
PRESENCE OF FOOT-AND-MOUTH DISEASE VIRUS IN THE PITUITARY AND CENTRAL NERVOUS SYSTEM OF EXPERIMENTALLY INFECTED CATTLE


Certain animal by-products for the production of pharmaceuticals and other products are imported into the United States from countries where foot-and-mouth disease (FMD) is enzootic. Among these animal by-products are certain components of the central nervous system (CNS), primarily the pituitary gland and the spinal cord.

Limited data are available on the presence and persistence of foot-and-mouth disease virus (FMDV) in the pituitary and CNS of cattle. This study was conducted in order to determine (1) is virus present in the pituitary and CNS of FMD-infected cattle and (2) if so, is it also present in the early clinical and convalescent stages thus increasing the risk of importing FMD as a result of importing CNS by-products containing FMDV.

REVIEW OF LITERATURE

Although no reference was found where FMDV had been isolated from the pituitary gland of cattle, an epizootic of FMD in England resulted from treating a cow with a commercially prepared pituitary extract. This extract had been produced on the mainland from cattle presumably in the early stages of infection and imported into England. Three additional vials from this lot of pituitary extract were recovered and tested in cattle and guinea pigs. All three contained FMDV.

Galloway isolated virus from the pituitary gland of guinea pigs infected with FMD. However, he was unable to show that the pituitary harbored virus longer than did the blood.

Several authors have indicated the involvement of the pituitary gland in animals infected with FMD based on clinical, histopathological and histochemical studies.

Most of these studies were conducted on cattle which showed signs of a late developing sequella to FMD commonly called "panting." This has also been referred to as a "heat intolerance syndrome," and "hairy cow syndrome." The typical signs shown by these "panters" include: hyperpnea especially when exposed to heat, a disturbed regulation of body temperature, skin lesions with hypertrichosis, disturbed metabolism resulting in either emaciation or obesity, a decrease in lactation, and disturbed reproductive physiology (anestrus, abortions, dead or weak calves).

From the Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Greenport, Long Island, New York.
Domanski and Fitko\textsuperscript{5} showed that "panting" resulted from a hormonal upset resulting from disorders of the endocrine system, especially the pituitary gland. Histopathological and histochemical techniques on emaciated "panters" showed a hypersecretion of thyroid-stimulating hormone (TSH) from the pituitary gland and a resulting hyperactive thyroid.\textsuperscript{5,11,12} The average weight of the pituitary glands from emaciated "panters" was significantly less than that of controls.\textsuperscript{5} Obese "panters" on the other hand showed a retention of TSH in the pituitary gland.\textsuperscript{5}

Nal\textsuperscript{14} reported multiple foci of lymphoid cells in the pituitary and other endocrine glands of cattle following FMD, and Fischer\textsuperscript{6} observed some histopathology in the hypothalamus and pituitary of swine 12 days after inoculation with FMD.

Minett\textsuperscript{11} in a study of over 4,000 cattle on military farms in India reported an incidence of 8.4 percent "panters" in European and crossbred cattle, and 2.4 percent in native cattle.

Kunter\textsuperscript{9} did a study on virus isolation from the CNS of cattle infected with FMD. In his study, samples from the cerebrum, cerebellum, medulla and spinal cord were pooled, then examined for virus by titration on cattle tongues. Virus was isolated from 14 of 43 CNS examined. In six animals, the individual components were tested for virus separately with two cerebrums and one cerebellum being positive. No virus was isolated from the spinal cords of these six animals. The highest titer found in the CNS in his experiments was $10^2$. Kunter concluded that the virus was probably not in the actual nervous tissue, but merely in the blood within the CNS. He also measured the pH of the CNS tissues tested and concluded that the low pH of the tissues tended to inactivate FMDV.

Wittman\textsuperscript{16} isolated virus from the brain of each of the three swine infected with FMD. The titers were $10^6.8$, $10^{5.2}$, and $10^{5.4}$ mouse LD\textsubscript{50}/gm. He was able to isolate virus from the last brain up to four days when stored at room temperature, and up to 27 days when stored at 4 C.

**MATERIALS AND METHODS**

**Viruses:** Six strains representing three types (A, O and C) of FMDV were used. These included four strains obtained originally from field cases in Argentina and passed two to four times in cattle; A-1 CANEFA,\textsuperscript{*} C-3 CANEFA, O-2 CANEFA and O-9 de Julio CANEFA. In addition, FMDV O-39 passed 86 times in cattle, and A-119 passed 89 times in cattle were used.

**Donors:** Eighteen grade Hereford steers 1-1/2 to two years old served as donor animals. These were inoculated with FMDV by the intradermolingual (I.D.L.) route in all but two cases. One steer (donor 3) was inoculated intramuscularly (I.M.) and another steer (donor 15) intraperitoneally (I.P.)

The donor steers were slaughtered at various stages of infection ranging from 12 hours to eight days post inoculation (HPI and DPI). The

\*Comision Asesora Nacional para la Erradicación de la Fiebre Aftosa.
donors were classified into three groups depending upon signs and lesions of FMD shown. These groups were designated as (1) early clinical—showing very slight signs and lesions, (2) clinical—showing acute signs and lesions, and (3) convalescent—showing normal temperatures, fair to good appetites and healing lesions. The number of donors in each group were three, seven, and eight respectively.

Collection of samples: Samples were obtained at slaughter from the following tissues: cerebrum, cerebellum, hippocampus, medulla, pineal body, pituitary gland, and lumbar spinal cord. Heparinized blood was collected for the determination of viremia. Cerebrospinal fluid (CSF) was also obtained from four donors.

After removing all meninges, the tissue samples were washed three times in a sterile buffered solution (LC fluid*). The tissues were then minced, placed in screw-capped vials, and frozen at -50 C. The pituitary glands from three donors (6, 7, 10) were divided into anterior and posterior portions and were frozen in separate vials.

Virus Isolation: After storage at -50 C for 1-30 days, the samples were thawed, ground with mortar and pestle with the aid of an abrasive, and sufficient LC fluid added to make a 1:10 suspension. After centrifugation at 880 xg for 20 minutes at 4 C, tenfold dilutions were prepared from the supernatant fluid with LC fluid.

Tests for the presence of FMDV were made by plaque assay in primary bovine kidney cell monolayer cultures as described by Bachrach, et al.2

Titration of most samples was also performed in suckling mice, using Rockefeller strain H white mice, five to eight days of age. Ten mice were inoculated with each dilution, each receiving 0.05 ml. I.P. Deaths were recorded for seven days, and the mouse LD_{50}/gm. was calculated from the seventh day reading using the method of Reed and Muench.15

Virus titration in steers was performed according to a previously described method.8

pH Determination: The pH of fresh tissues from eight donors was determined immediately after collection. The pH readings obtained were evaluated in order to determine if virus inactivation might occur within the tissues because of a low pH.

RESULTS

All donor steers inoculated I.D.L. developed signs of FMD within 24 HPI. Steers one and two were slaughtered 12 and 16 HPI. Both had normal temperatures and showed no external signs of illness. The only lesions of FMD observed at necropsy were one cm. vesicles on the tongue at the sites of inoculation. The steer inoculated I.M. (donor three) showed no

*Hanks' buffered salt solution. 0.5 percent lactalbumin hydrolysate, two percent bovine serum, and antibiotics (100 units/ml. each of penicillin, dihydrostreptomycin, and polymyxin, and 25 units/ml. of mycostatin).
## TABLE I
Titer of Foot-and-Mouth Disease Virus in the Blood, Pituitary, Pineal Body and Spinal Cord of Experimentally Infected Cattle

<table>
<thead>
<tr>
<th>Donor</th>
<th>Virus</th>
<th>DPI</th>
<th>TC Mice</th>
<th>Pituitary†</th>
<th>Pineal Body</th>
<th>Spinal Cord</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood</td>
<td>Pituitary†</td>
<td>Pineal Body</td>
</tr>
<tr>
<td>EARLY CLINICAL FMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>O-9</td>
<td>1/2</td>
<td>5.1</td>
<td>4.2</td>
<td>2.1</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>O-9</td>
<td>2/3</td>
<td>5.5</td>
<td>4.3</td>
<td>3.3</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>C-3</td>
<td>2*</td>
<td>5.2</td>
<td>4.5</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>CLINICAL FMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>O-9</td>
<td>2</td>
<td></td>
<td>5.6</td>
<td>6.8</td>
<td>6.0‡</td>
</tr>
<tr>
<td>5</td>
<td>O-9</td>
<td>2</td>
<td>5.6</td>
<td>5.7</td>
<td>6.1</td>
<td>6.4</td>
</tr>
<tr>
<td>6</td>
<td>O-2</td>
<td>2</td>
<td>5.4</td>
<td>4.4</td>
<td>4.8</td>
<td>3.4</td>
</tr>
<tr>
<td>7</td>
<td>O-39</td>
<td>2</td>
<td>P</td>
<td>2.0</td>
<td>—</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>A-1</td>
<td>3</td>
<td>4.4</td>
<td>4.2</td>
<td>6.0</td>
<td>5.3‡</td>
</tr>
<tr>
<td>9</td>
<td>A-119</td>
<td>3</td>
<td>5.2</td>
<td>3.8</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>O-2</td>
<td>4</td>
<td>2.8</td>
<td>5.7</td>
<td>6.0</td>
<td>5.1</td>
</tr>
<tr>
<td>CONVALESCENT FMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>O-9</td>
<td>6</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>5.2</td>
</tr>
<tr>
<td>12</td>
<td>O-9</td>
<td>6</td>
<td>N</td>
<td>N</td>
<td>3.4</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>O-2</td>
<td>6</td>
<td>N</td>
<td>—</td>
<td>3.3</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>O-39</td>
<td>6</td>
<td>N</td>
<td>—</td>
<td>N</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>C-3</td>
<td>8**</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>16</td>
<td>C-3</td>
<td>8</td>
<td>N</td>
<td>—</td>
<td>N</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>O-9</td>
<td>8</td>
<td>N</td>
<td>—</td>
<td>N</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>A-1</td>
<td>8</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N‡</td>
</tr>
<tr>
<td>Total positive</td>
<td>10/18</td>
<td>11/18</td>
<td>3/17</td>
<td>4/18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DPI = days post inoculation  
P = positive  
N = negative  
— = not tested  
Mice = log mouse LD50/gm.  
TC = log PFU/gm.  
* = intramuscular inoculation  
** = intraperitoneal inoculation  
† = donors 6, 7, and 10, numerator = anterior pituitary, denominator = posterior pituitary  
‡ = also tested in steers: (1) pituitary, donors 4, 8, and 12 = 10^6.0, 10^5.2, 10^3.2  
bovine ID50/gm., donor 18 = N; (2) Spinal cord, donors 3 and 18 both P.

Clinical signs of illness prior to slaughter (45 HPI) other than a temperature of 106.8°F. At necropsy, this steer showed three very small unruptured vesicles on the tongue, one small unruptured vesicle on one foot, and very slight lesions of the rumen epithelium. The steer which received
The intraperitoneal inoculation (donor 15) developed signs of FMD 72 HPI. The FMDV titers in blood, pituitary, pineal body, and spinal cord from the 18 donor steers tested are listed (Table I).

Viremia was present in all early clinical and clinical donors, but none of the convalescent donors were viremic.

The titers obtained from the anterior and posterior pituitaries were similar from the three donors in which the pituitaries were divided and tested separately.

The presence of FMDV in the spinal cords of donor three (2 DPI) and donor 18 (8 DPI) was confirmed in steers.

The number of samples yielding virus and the number of samples tested during the early clinical, clinical and convalescent stages of infection are listed for each of the nine tissues tested (Table II). The highest titer obtained from each tissue is also listed.

### TABLE II

Presence of Foot-and-Mouth Disease Virus in the CNS Components of Cattle During the Various Stages of Infection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Highest titer*</th>
<th>Early Clinical</th>
<th>Clinical</th>
<th>Convalescent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5.6</td>
<td>3/3**</td>
<td>7/7</td>
<td>0/8</td>
<td>10/18</td>
</tr>
<tr>
<td>Pituitary</td>
<td>6.8</td>
<td>3/3</td>
<td>5/7</td>
<td>3/8</td>
<td>11/18</td>
</tr>
<tr>
<td>Pineal Body</td>
<td>4.3</td>
<td>1/3</td>
<td>1/6</td>
<td>1/8</td>
<td>3/17</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>3.2</td>
<td>2/3</td>
<td>1/7</td>
<td>1/8</td>
<td>4/18</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>2.5</td>
<td>0/3</td>
<td>1/7</td>
<td>0/8</td>
<td>1/18</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>N</td>
<td>0/3</td>
<td>0/5</td>
<td>0/3</td>
<td>0/11</td>
</tr>
<tr>
<td>Medulla</td>
<td>N</td>
<td>0/3</td>
<td>0/5</td>
<td>0/3</td>
<td>0/11</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>N</td>
<td>0/2</td>
<td>0/3</td>
<td>0/1</td>
<td>0/6</td>
</tr>
<tr>
<td>CSF</td>
<td>3.4</td>
<td>1/1</td>
<td>2/2</td>
<td>0/1</td>
<td>3/4</td>
</tr>
</tbody>
</table>

* = log PFU/gm.

** = Numerator = number of samples containing virus; denominator = number of samples tested.

From the 18 donors tested, virus was isolated from 11 pituitary glands, three pineal bodies, four spinal cords, and one cerebrum. Virus was also isolated from three of the four samples of CSF tested.

Virus was not isolated from the cerebellum, medulla, or hippocampus, with 11, 11, and six samples being tested, respectively.

The virus isolated from the pineal body of the convalescent steer (donor 16, C-3 virus, 8 DPI) was confirmed to be type C of FMDV by neutralization tests.4

The pH readings obtained from the CNS components of the eight steers checked are listed (Table III). The average pH of the pituitary glands was slightly higher than that of other CNS components. The lower pH values would tend to inactivate FMDV1 as suggested by Kunter.9 However, to explain the virus distribution found in the CNS components in this experiment by pH alone would require further studies.
TABLE III

pH of CNS Components of FMD Infected Steers

<table>
<thead>
<tr>
<th>Donor</th>
<th>Virus</th>
<th>DPI</th>
<th>Pituitary</th>
<th>Spinal Cord</th>
<th>Cere-brum</th>
<th>Cere-bellum</th>
<th>Medulla</th>
<th>Pineal Body</th>
<th>Hippo-campus</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>C-3</td>
<td>2</td>
<td>7.0*</td>
<td>6.6*</td>
<td>5.7</td>
<td>6.0</td>
<td>5.8</td>
<td>-*</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>O-9</td>
<td>2</td>
<td>6.9</td>
<td>6.7</td>
<td>6.2</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4*</td>
<td>6.1</td>
</tr>
<tr>
<td>8</td>
<td>A-1</td>
<td>3</td>
<td>6.6*</td>
<td>6.3</td>
<td>5.5</td>
<td>5.7</td>
<td>6.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>O-2</td>
<td>4</td>
<td>6.8*</td>
<td>6.5</td>
<td>6.2</td>
<td>5.8</td>
<td>6.3</td>
<td>5.9</td>
<td>6.2</td>
</tr>
<tr>
<td>12</td>
<td>O-9</td>
<td>6</td>
<td>6.1*</td>
<td>6.0</td>
<td>6.1</td>
<td>5.9</td>
<td>6.0</td>
<td>-</td>
<td>5.8</td>
</tr>
<tr>
<td>11</td>
<td>O-9</td>
<td>6</td>
<td>6.8*</td>
<td>6.8</td>
<td>6.7</td>
<td>6.6</td>
<td>6.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>C-3</td>
<td>8</td>
<td>6.7</td>
<td>6.8</td>
<td>6.6</td>
<td>6.8</td>
<td>6.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>A-1</td>
<td>8</td>
<td>6.8</td>
<td>6.5*</td>
<td>6.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average pH</td>
<td>6.7</td>
<td>6.5</td>
<td>6.2</td>
<td>6.2</td>
<td>6.2</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* = FMDV isolated from tissue  
- = pH not tested

DISCUSSION

The results obtained show that the virus of foot-and-mouth disease was present in the pituitary gland of cattle during early clinical, clinical and early convalescent stages of the disease. The virus titer of four of the five positive pituitary glands during the clinical stage was higher than the virus titer of the corresponding blood. This high titer plus the persistence of the virus in the pituitary after viremia strongly indicate that the presence of the virus is not merely a result of the blood present within the gland. This may indicate that concentration or replication of FMDV does occur within the pituitary gland.

Of the remaining components of the CNS, the spinal cord, pineal body and cerebrum contained FMDV in some infected cattle but less frequently, and in lower concentration, than in the pituitary. It is possible that other components could occasionally contain virus.

The results of this experiment differ from Kunter's in that virus was found in the spinal cord more often than in any CNS material other than the pituitary and CSF. Also, one spinal cord was shown to contain virus 8 DPI, or three to four days after the end of viremia.

All pituitary hormone extraction processes do not inactivate the virus of FMD as evidenced by the outbreak of FMD caused by a commercial pituitary extract in England. The exact extent of the hazard could only be ascertained following a study of the effect of each pharmaceutical process on FMDV.

SUMMARY

Foot-and-mouth disease virus (FMDV) was present in high titers in the pituitary gland of cattle during the early clinical, clinical and early convalescent stages of the disease. The highest titer obtained was
10^6.8 PFU/gm. The titers were equal to or higher than those in the blood, and the virus persisted in the pituitary one to two days after viremia.

Virus was also isolated from the spinal cord, pineal body, cerebrum, and cerebrospinal fluid but less frequently, and with lower concentrations, than from the pituitary. Virus was not isolated from the cerebellum, medulla, or hippocampus.

REFERENCES

STUDIES ON THE TRANSMISSION OF AFRICAN SWINE FEVER VIRUS BY ARTHROPODS

W. P. Heuschele* and L. Coggins**

INTRODUCTION

The sporadicity and scattered geographic distribution of outbreaks of African swine fever (ASF) in East Africa led to suspicions by earlier workers on this disease that an arthropod vector might be important in the transmission of the causative virus.\textsuperscript{11,12} This hypothesis is supported further by the experience that contact transmission of African swine fever virus (ASFV) from known wart hog carriers to susceptible domestic pigs and wart hogs has been rarely accomplished experimentally.\textsuperscript{5,11,12}

Montgomery\textsuperscript{11} failed to effect transmission of ASFV by feeding \textit{Haemotopinus suis} on ASF-infected pigs followed by their feeding on susceptible pigs.

Recently, Botija\textsuperscript{1} found ASFV present in the tick, \textit{Ornithodoros erraticus}, in piggeries where ASF outbreaks have occurred and established that these reservoir ticks can transmit this virus as long as six to 12 months after their last meal on an ASF-infected animal.\textsuperscript{1,2}

In Kenya, the Argasidae tick, \textit{Ornithodoros moubata}, is a common inhabitant of ground burrows occupied by wart hogs, porcupines, and the aardvark.\textsuperscript{9,13,14} When present in any of these habitats, several thousand are found in a relatively small area.\textsuperscript{9,13,14} The fact that this is a multiple host tick makes it ideally suited as a potential vector of ASF. Nymphs and adults of both sexes feed on animals.\textsuperscript{9} It has already been established as an important vector of human relapsing fever.\textsuperscript{9,13,14}

Studies were therefore conducted on this tick and on \textit{Haemotopinus suis} to further evaluate their capability as potential ASFV vectors.

MATERIALS AND METHODS

Several hundred hog lice (\textit{H. suis}) were collected from normal swine at a local abattoir. These were maintained on a normal pig in an isolation stall surrounded by a water barrier. Transmission trials were conducted

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TRANSMISSION OF ASF VIRUS BY ARTHROPODS

by placing a number of lice in a cloth bag which was taped over the ear of pigs upon which they were to feed.

Ornithodoros moubata ticks were collected from a number of wart hog burrows in the Ewaso Ngiro Plains area, 22 mile west of Lake Magadi, Kenya, an area with a large wart hog population where DeTray et al.7 found five out of nine wart hogs to be ASFV carriers. Ticks were colonized in gauze-capped bottles and were given maintenance feedings on the ears of normal rabbits when not being used in transmission trials. Feedings on pigs were made by placing a number of ticks in a bottle and holding the inverted mouth of the bottle tightly against the skin behind the ear or in the groin of the pig until all or most of the ticks were attached and feeding. The bottle was then removed, and engorged ticks were removed manually as they detached themselves, usually within 30 minutes after attachment.

Virus assays of pig blood and tissues and ground suspensions of lice and ticks in MEM* were conducted in swine buffy coat cultures (BC) according to methods previously described.8,10

Yorkshire-type pigs were used in all experiments and feeding on infected pigs were made at the peak of fever reaction to inoculation with ASFV. Blood/virus titers of these pigs were usually determined at the time of such tick feedings.

Several different field isolates of ASFV were used to inoculate pigs. These were named after the geographic locality where first obtained, since no distinct immunological types or strains of ASFV have as yet been established.6

Before transmission trials were conducted, 100 O. moubata nymphs were randomly selected from the total collection and tested for ASFV by inoculating BC cultures with a 10 percent s/v ground tick suspension.

Haematopinus suis Transmission Experiments. (1) Sixty-eight H. suis lice were allowed to feed one hour on pig 1122, infected with ASFV (Tengani). At this time, the pig had a blood/virus titer of $10^7$HAU$_{50}$/ml.** The lice were held overnight and then allowed to feed one hour on susceptible pig 1083. Following this procedure, the virus content of the lice was assayed. (2) Ninety-eight lice were fed three hours on pig 1126, infected with ASFV (Tengani) and having a blood/virus titer of $10^7$HAU$_{50}$/ml. After being held overnight, 83 lice were still alive and were allowed to feed for 23 hours on susceptible pig 1115. Virus content of lice was assayed after this feeding. (3) Twenty-eight lice were fed 24 hours on pig 1143, infected with ASFV (Tengani). The lice were transferred immediately thereafter to susceptible pig 1152 and allowed to feed 24 hours. Virus content of lice was assayed after this feeding.

Ornithodoros moubata transmission Experiments. (4) Forty O. moubata nymphs were allowed to feed on pig 1072, infected with ASFV (Magadi wart hog 6-64), having a blood/virus titer of $10^7$HAU$_{50}$/ml. at this

*Eagle's Minimum Essential Medium
**50 percent Hemadsorption units
time. An aliquot of five nymphs was assayed for virus content three days later. Seven days later, 10 of the remaining nymphs were fed on the ear of susceptible pig 1086. (5) Fourteen days after obtaining their infectious meal on pig 1072, two groups of 10 nymphs were each fed respectively on susceptible pigs 1137 and 1138. (6) Seventy-three days after feeding on infected pig 1072, five nymphs were assayed for virus content. (7) Ninety nymphs and 40 adult *O. moubata* were fed on pig 1196, infected with ASFV (Uganda). (8) Fourteen days later, 85 of these nymphs fed on susceptible pig 1209 and 35 of the adults fed on susceptible pig 1146 in the same stall. Following these feedings, both groups of ticks were ground and 0.1 ml. of the 10 percent w/v suspension of each was inoculated into BC cultures. (8) Thirty-three *O. moubata* ticks were allowed to feed on pig 1209, infected with ASFV (Uganda) by ticks in the previous experiment. One tick from this group was then ground and inoculated into BC cultures. (9) The remaining ticks were held 21 days, at which time one was dead and the surviving 20 were allowed to feed on susceptible pig 1179. Following this procedure, one tick was checked for ASFV in BC culture as before. Culture fluid from four BC cultures inoculated with this tick suspension was inoculated into susceptible pig 1223.

**RESULTS**

The results of experimental transmission of ASFV by lice and ticks are summarized in Table I.

The random sample of 100 *O. moubata* nymphs collected from wart hog burrows was found free of ASFV.

The 68 lice (*H. suis*), which fed on infected pig 1122, had a virus content of $10^{3.5}$ HAU$_{50}$/gm. on subsequent assay. Lice assayed after feeding on infected pigs 1126 and 1143 contained no ASFV. No transmission of ASFV occurred when any of the groups of lice fed upon susceptible pigs following their feeding on infected pigs.

Five *O. moubata* nymphs contained $10^{6.25}$ HAU$_{50}$/gm. of ASFV three days after feeding on infected pig 1072.

Pig 1086 was found dead the day after 10 *O. moubata* nymphs, which fed on infected pig 1072 seven days earlier, were allowed to feed on its ear. Death was attributed to shock and not ASF. However, hemadsorption was observed in cultures inoculated with a suspension of the prescapular lymph node whose afferent lymphatic vessels drained the area where the infected ticks had fed. This finding established that viral transmission had been effected by the ticks. Other samples from this pig, which were tested for ASFV without hemadsorption, were the mediastinal, inguinal, cervical, gastric and mesenteric lymph nodes, the lung, tonsil, stomach, spleen, liver, and kidney tissues and whole blood.

No transmission of ASFV to pigs 1137 and 1138 was effected by ticks held 14 days after feeding on infected pig 1072.

In experiment five, pig 1209 showed fever (104.6 F.) three days after exposure to 85 nymphs of *O. moubata*, which had fed on infected pig 1196 14 days before. This pig died on the tenth day after exposure to the
TABLE I
Transmission of African Swine Fever Virus (ASFV) from Infected to Susceptible Pigs by Arthropods:
Experiments Using *Haematopinus suis* Lice and *Ornithodoros moubata* Ticks

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Infected Arthropod to which exposed</th>
<th>Interval after arthropod obtained infected blood meal</th>
<th>ASFV present in arthropod</th>
<th>Reaction of susceptible pig after exposure to arthropod</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1083</td>
<td><em>H. suis</em></td>
<td>68</td>
<td>23 hours</td>
<td>$10^{3.5}$ HAU$_{50}$/gm.</td>
</tr>
<tr>
<td>1115</td>
<td><em>H. suis</em></td>
<td>98</td>
<td>24 hours</td>
<td>None</td>
</tr>
<tr>
<td>1152</td>
<td><em>H. suis</em></td>
<td>28</td>
<td>Immediate</td>
<td>None</td>
</tr>
<tr>
<td>1086</td>
<td><em>O. moubata</em></td>
<td>10</td>
<td>7 days</td>
<td>$10^{6.5}$ HAU$_{50}$/gm.</td>
</tr>
<tr>
<td>1137</td>
<td><em>O. moubata</em></td>
<td>10</td>
<td>14 days</td>
<td>N.D.**</td>
</tr>
<tr>
<td>1138</td>
<td><em>O. moubata</em></td>
<td>10</td>
<td>14 days</td>
<td>N.D.</td>
</tr>
<tr>
<td>1209</td>
<td><em>O. moubata</em></td>
<td>85</td>
<td>14 days</td>
<td>$10^{2}$ HAU$_{50}$/gm.</td>
</tr>
<tr>
<td>1146</td>
<td><em>O. moubata</em></td>
<td>35</td>
<td>14 days</td>
<td>$10^{2}$ HAU$_{50}$/gm.</td>
</tr>
<tr>
<td>1179</td>
<td><em>O. moubata</em></td>
<td>31</td>
<td>21 days</td>
<td>$10^{2}$ HAU$_{50}$/gm.</td>
</tr>
</tbody>
</table>

*50 percent Hemadsorption units

**Not determined
infected ticks and had ASF lesions at necropsy. Hemadsorption was produced in BC cultures inoculated with spleen suspension from this pig, and ASF/AGDP\textsuperscript{3} antigen was demonstrated in the inguinal, gastric, iliac, mesenteric and prescapular lymph nodes, the liver, lung, thymus, kidney, spleen, and tonsil tissues, and the urine.

Pig 1146 was febrile on the ninth day after exposure to 35 adult ticks, which fed 14 days before on infected pig 1196. This pig died on the fourteenth day and had ASF lesions at necropsy. Hemadsorption was produced in BC cultures inoculated with spleen suspension from this animal. The ASF/AGDP antigen was demonstrated in pancreas, bile, spleen, liver, gall bladder, kidney, and tonsil samples, and mesenteric, prescapular, inguinal, iliac and gastric lymph nodes.

Thirty-two ticks failed to transmit ASFV to pig 1179 21 days after feeding on pig 1209, which had a temperature of 106 F. at five days after exposure to infected ticks. In this case, ASFV was demonstrated in one tick immediately after its feeding on pig 1209 and in one tick 21 days later. Culture fluids from BC cultures inoculated with a suspension of this latter tick caused ASF when inoculated into a susceptible pig.

A suspension of five \textit{O. moubata} nymphs, prepared 73 days after they had fed on infected pig 1072, did not produce hemadsorption when inoculated into BC cultures.

**DISCUSSION**

The results of hog lice (\textit{H. suis}) feeding on ASF-infected pigs agree with the observations of Montgomery,\textsuperscript{11} who also failed to obtain transmission of ASF by this arthropod. The finding of virus in the lice after feeding in experiment one suggests that additional studies, using larger numbers of lice and longer feeding periods, might be worthwhile.

The finding of ASFV in regional lymph nodes, whose afferent lymphatic vessels drained the area where infected ticks had fed, indicates that virus transmission was effected by the ticks in experiment four.

The death of pig 1209 from ASF in experiment five confirms the capability of \textit{O. moubata} to transmit ASFV. Contact transmission of ASFV from pig 1209 to 1146 in experiment six cannot be ruled out.

It appears from these data that transmission of ASFV from infected to susceptible pigs by \textit{O. moubata} may require large numbers of ticks. Botija\textsuperscript{2} has made similar observations using \textit{O. erraticus}. However, it is not unusual to find several thousand ticks on an animal entering a wart hog burrow or in piggeries infested with these ticks.

The failure to demonstrate ASFV in a random sample of 100 \textit{O. moubata} nymphs obtained from wart hog burrows leaves the question still open of their role in the epizootiology of ASF in wart hogs. Out of 13 wart hogs, collected at the same time and location as the tick collections, eight were ASFV carriers. However, virus was demonstrated in the blood of

*Agar gel diffusion precipitin
only three animals. The remaining five carriers had demonstrable virus only in lymph nodes and spleen.

The ASFV was demonstrated in *O. moubata* at 21 but not at 73 days after exposure to infected pigs. *Ornithodoros erraticus* has retained ASFV in excess of six months after such exposure.²

It might, therefore, be postulated that viremia in ASF-carrier wart hogs is transient or sporadic; hence, finding infected ticks in their habitat would depend on the status of viremia in indigenous wart hog carriers at the time.

**SUMMARY**

The capability of the hog louse, *Haematopinus suis*, and the African Argasidae tick, *Ornithodoros moubata*, to transmit African swine fever virus was investigated. Virus was demonstrated in lice one day after feeding on an ASF-infected viremic pig; however, transmission of ASFV was not accomplished by feeding these lice on susceptible pigs.

Transmission of ASFV to susceptible pigs was effected by 10 *O. moubata* nymphs seven days after an infected blood meal and by 85 nymphs 14 days after such a meal. Two pigs failed to become infected by ASFV when fed upon by 10 *O. moubata* nymphs 14 days after they had obtained an infected blood meal.

Transmission of ASFV was not accomplished when 31 ticks were fed on a susceptible pig 21 days after obtaining an infected blood meal. Virus was, however, demonstrated in a tick from this group at 21 days after its meal by hemadsorption in cultures inoculated with tick suspension, and subsequent ASF infection in a pig inoculated with this culture fluid. A suspension of 100 *O. moubata* nymphs, randomly sampled from several wart hog burrows in an area in which approximately 60 percent of wart hogs examined were ASFV carriers, did not produce hemadsorption when inoculated into swine buffy coat cultures.

It is concluded that *O. moubata* is capable of transmitting ASFV but that large numbers of ticks may be required.

The finding of ASFV in ticks at 21 days but not at 73 days after an infected blood meal suggests that this species retains the virus for a relatively short period of time.

**ACKNOWLEDGEMENT**

The authors are grateful to Mr. Denis Zaphiro, District Game Warden, Kajiado, Kenya, for permission to carry out the tick collections described. We thank Mr. B. Highton, Medical Research Laboratory, Nairobi, Kenya, for valuable advice and assistance on collection of the tick and maintenance of colonies. Our thanks also to Miss J. B. Walker, East African Veterinary Research Organization, Muguga, Kenya, for her considerable advice and assistance in tick handling and maintenance and identification.

The technical assistance of Mr. Geoffrey Chege and William T. Onyango is gratefully acknowledged.
REFERENCES

REPORT OF THE COMMITTEE ON ANIMAL
VIRUS CHARACTERIZATION


During the past year, considerable progress has been made in the activities of the Committee for Non-Primate Animal Virus Characterization. The data processing center, which previously existed in Berkeley, California under the direction of Dr. Jacob Traum, has been transferred to the Institute for Comparative Biology of the San Diego Zoological Society to be directed by Dr. Charles J. York. These activities of the Committee are being supported by an NIH grant, as heretofore. Two meetings have been held by the Committee during the past year—one in April 1965 and one in October—to analyze and evaluate the virus characterization data so far submitted, and to project the future activities of the group. The discussions encompassed the following points:

1. Data Collection

Over 110 virus characterization questionnaires have now been returned to the Committee. These represent material on approximately 90 viral agents, the majority of which are new isolates. The IBM system, because of its flexibility, has been chosen to record and cross-index the mass of information which is being collected. It is hoped that the transfer of information from the virus characterization questionnaires to the IBM card system will be accomplished by next spring.

2. Virus Classification

One of the major aims of the Committee is to promote systematic classification of viruses. Although the classification system presently in use has not received the approval of the International Microbiological Societies and may be drastically revised in the near future, the Committee nevertheless feels that it behooves the investigators in the animal virus field to align themselves with the existing system wherever possible. Review of the information accumulated at our center and of the published data indicates that a considerable number of viruses could be assigned to the presently established taxonomic groups. The majority of the viruses, however, are still inadequately characterized and require additional studies.
before taxonomic classification will be possible. It also appears likely that certain viral agents have characteristics sufficiently divergent from the presently described viral groups to justify establishment of new taxonomic groupings. The Committee is undertaking a provisional classification of the better characterized animal viruses and will offer this information for the consideration of the Provisional Virus Nomenclature Committee of the International Microbiological Societies at the forthcoming international congress in Moscow. To expedite this work, several subcommittees composed of the members of the Committee were appointed during the April meeting with the responsibility of collecting data on specific viral groups.

3. Reference Reagents

Definitive characterization and classification of viruses entail detailed and precise comparison of similar agents obtained from different parts of the world. Such comparisons can be accomplished only through the use of properly produced reference reagents. In view of this, the Committee has devoted considerable attention to the problem of reference reagent development. In the opinion of the Committee, the data so far received by the collection center offers sufficient basis to begin the systematic selection of provisional reference viruses that could be used in the production of reference antigens and antisera, as well as for other research purposes. The selection of these reference viruses will be carried out in accordance with the provisions previously stated by the Committee in its 1964 progress report. The provisional selection of reference viruses will be carried out by the working subcommittees and their recommendations will then be submitted for the approval of the Committee as a whole and for review by the counterpart Eastern Hemisphere Committee. It is contemplated that when full agreement is reached on the selected viral agents, the scientific community will be informed of the actions of the Committee through publication.

In order to expedite the collection of the necessary characterization data on the older well recognized viruses, the members of the working subcommittees are to accumulate such information by making direct contact with investigators especially well versed in specific areas of viral research. Here good progress has already been made since the April meeting, particularly in the areas of bovine, porcine and canine viruses. However, considerable time will be required before this task is completed.

The activities of the Eastern and Western Committees on Non-Pri-mate Animal Virus Characterization are being coordinated by appropriate meetings of representatives of the two organizations. Contact with committees dealing with the human viruses is being maintained through the auspices of WHO and participation in international meetings.*

*For example, to further the international scope of this activity, two members of the Committee attended the annual meeting of the Directors of the World Health Organization International Virus Reference Laboratories.
It has always been recognized that for the virus characterization effort to be successful, the program has to be international in scope. To date the existing committees do not fully reflect this intent. Accordingly, efforts will now be directed towards inviting competent virologists from other parts of the world to become associated with this activity in order to make this a truly international effort.
The etiology of bovine shipping fever has been a point of discussion for several years. Early investigations were concerned with the role of Pasteurella sp. The report of Reisinger, Heddleston, and Manthei in 1959 on the isolation of a Myxovirus Parainfluenza-3 has stimulated research on this viral agent and the role it plays in shipping fever. Based upon serologic, clinical, and experimental studies, this virus has been recently looked upon as the most important etiological agent of bovine shipping fever in the United States.

The purpose of this report is to present data on the immunity calves obtained when vaccinated with a combination inactivated bovine Parainfluenza-3 (PI-3) vaccine and Pasteurella hemolytica-Pasteurella multocida bacterin. Immunity was measured by hemagglutination inhibition (HI) antibody levels and resistance to an experimental challenge containing PI-3 and Pasteurella.

The experimental shipping fever vaccine contained Reisinger's SF-4 strain of bovine PI-3, Heddleston's bovine strains of P. multocida (P-1062) and P. hemolytica (P-1148), and was formalin inactivated with an adjuvant added.

There were 32 calves involved in this study and both calves and their dams were bled soon after birth for HI titer determinations. A few calves were bled before they had an opportunity to obtain any colostrum. The results of these blood samples are presented in Table I. As expected, no HI titer was observed before colostrum. Following colostrum the HI titers became apparent and correlated with the HI titers of their dams.

At four to 10 weeks of age, the calves were vaccinated with blackleg and malignant edema bacterin and 18 of the calves were vaccinated with

TABLE I

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Calves</th>
<th>Dams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Colostrum</td>
<td>After Colostrum</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>30.5*</td>
</tr>
<tr>
<td></td>
<td>0 - 320</td>
<td>0 - 160</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>0 - 160</td>
<td>18.7</td>
</tr>
</tbody>
</table>

*Geometric Mean

Veterinary Research Department, Agricultural Research Center, Eli Lilly and Company, Greenfield, Indiana.
the experimental shipping fever vaccine. These 18 calves were revacci-
nated with the shipping fever vaccine when they were three to four and one  
half months old. The reason for this time interval between vaccinations  
was to study the effects of a vaccination schedule which would be more  
compatible with management generally practiced by stockmen. Other  
experimental studies with an inactivated shipping fever vaccine such as  
this has suggested that two vaccinations at a three-week interval just  
prior to shipping would induce the most satisfactory level of immunity.\textsuperscript{2,10}

The experimental challenge consisted of Reisinger's SF-4 strain of  
bovine PI-3 and Heddleston's bovine strains of \textit{P. hemolytica} and \textit{P. multocida} which were given to the animals as an aerosol after the calves  
had been stressed. Stress was accomplished by enclosing the animals in  
a heated room for 12 hours then trucking them in inclement weather with  
periodic spraying of water and finally leaving them on the truck overnight  
before challenging.\textsuperscript{7}

Clinical observations, blood samples and nasal swabs were collected  
daily for two weeks following challenge. Blood serum samples for HI de-
terminations were obtained at various time intervals throughout the  
course of this study. There were no detectable changes in the hematocrit,  
hemoglobin and white blood cell counts during the two-week period. The  
hemagglutination inhibition geometric mean titers (GMT) following vac-
cination and experimental challenge are presented in Table II. The GMT  
following challenge rose dramatically in the vaccinated animals as com-
pared to the unvaccinated controls and can be interpreted as an anamnestic  
response to the experimental challenge. The GMT of 112.9 in the vaccin-
ated animals as compared to 26.8 in the control group was found to be a  
significant difference at the 0.05 probability level.

The daily mean temperature differences between the vaccinated and  
unvaccinated animals are presented in Graph 1. The temperatures were  
higher and persisted longer in the control group when compared to the  
vaccinated animals. An analysis of variance showed the differences to be  
significant at the 0.01 or 0.05 probability levels on the third to sixth day  
post challenge.

The \textit{Pasteurella} and PI-3 isolations were made from nasal swabs  
taken from both groups of animals. The PI-3 isolants were characterized

\begin{table}
\centering
\caption{Geometric Mean HI Titers Following Vaccinations and Challenge}
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Time Blood Samples Taken*} & 1 & 2 & 3 & 4 \\
\hline
Vaccinates & 14.3 & 17.5 & 112.9 & 246.7 \\
Controls & 10.2 & 10.1 & 26.8 & 105.4 \\
\hline
\end{tabular}
\end{table}

1 = 3 months after 1st vaccination
2 = 7 days after 2nd vaccination
3 = 7 days post challenge
4 = 14 days post challenge
by hemadsorption, serum neutralization and cytopathogenic changes on primary bovine embryonic kidney tissue culture monolayers. Though viral isolations were made in both groups of animals, shedding persisted two to three days longer in the control group as presented in Table III.

Six calves (two vaccinates and four unvaccinated controls) died during the course of the experiment. One vaccinated calf died during the stress period and post-mortem revealed an extensive hepatic abscess. The other

**TABLE III**

PI-3 Isolation Following Challenge

<table>
<thead>
<tr>
<th>I. Percent of Calves Shedding PI-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
</tr>
<tr>
<td>Controls</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Days PI-3 Shed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>1 Day</td>
</tr>
<tr>
<td>Vaccinates</td>
</tr>
<tr>
<td>Controls</td>
</tr>
</tbody>
</table>

* Significant differences between groups
   * Probability level = 0.01
   ** Probability level = 0.05

Graph 1. Pyrexia Following Challenge in Vaccinates and Controls (Mean Temperatures).
vaccinated calf died after challenge and there were no gross or micro-
scopic lesions observed which were indicative of pneumonia. All four 
control calves that died showed varying degrees of pneumonia upon 
necropsy. Two of the animals had severe lung lesions characterized by 
consolidation, fibrin formation and emphysema.

Post-mortem lung examinations of animals sacrificed at the end of 
this experiment as well as those animals dying following challenge were 
categorized for sake of comparison. No lung involvement or very few 
isolated, small, focal areas were considered normal lungs. One or two 
lobes of the lung involved with some consolidation was considered a mod-
erate pneumonia. Several lobes involved with consolidation, abscessation, 
fibrin formation, edema and or emphysema, classified the lung as severe-
ly affected. Table IV presents the results of this classification. It would 
appear that the incidence and severity of pneumonic lesions has been re-
duced by vaccination.

Microscopic sections were also categorized for comparison. A lung 
section showing very little change or some cellular infiltration without 
loss of alveolar structure was considered normal to mild. Severe changes 
were indicated by abscessation, loss of alveolar structure and massive 
cellular infiltration. Table V presents the results of this comparison in 
these two groups of animals. Again, vaccination markedly reduced the 
incidence of cellular changes indicative of a severe pneumonia.

Based upon the results of the several parameters studied, an inacti-
vated shipping fever vaccine has been prepared which will induce a satis-
factory level of immunity against a severe experimental challenge. Other 
experimental data have indicated that this vaccine has induced a good pro-	ection against a natural outbreak of shipping fever. Excellent safety data 
and serologic conversions have been obtained in 923 animals in nine field 
trials.³

<table>
<thead>
<tr>
<th>TABLE IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross Signs of Pneumonia Between Vaccinates and Controls</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Lungs</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>(1/17 Severe)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results of Microscopic Lung Examinations</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Changes</td>
</tr>
<tr>
<td>Normal to Mild</td>
</tr>
<tr>
<td>Severe</td>
</tr>
</tbody>
</table>
SUMMARY

An inactivated vaccine including Parainfluenza-3, *Pasteurella multocida* and *Pasteurella hemolytica* induced good protection in calves as evidenced against a severe experimental shipping fever challenge. At four to 10 weeks of age the calves were first vaccinated and were revaccinated at weaning age. The vaccinated animals showed significantly higher HI titers, lower temperatures, and fewer lung lesions when compared to the control animals.

REFERENCES

The Committee on Biologics was informed of significant changes in the regulation of Veterinary Biologics at the Federal level. Reorganizational changes within the Agricultural Research Service have been made and two new divisions created. These are the Animal Health Division, formerly Animal Disease Eradication, and the Division of Veterinary Biologics, formerly Animal Inspection and Quarantine. The Division of Veterinary Biologics, under the directorship of Dr. John Hejl has four sections: Licensing and Permits, Field Operations, Laboratory (National Animal Disease Laboratory) and Technical Analysis. The Field Operations program is based at National Animal Disease Laboratory and replaces the previous field inspection stations. Field Operations and Laboratory are organized by disciplines, i.e., large animal, small animal, poultry and anaerobes. The key difference in the new program is that the resident inspector who was responsible for all products in a firm, has been replaced by specialists who will be responsible for one area, for example, large animal biologics across the United States.

New regulations have been promulgated concerning the use of substances that might leave residues in the animal. These regulations have necessitated the closer cooperation of the Veterinary Biologics Division, Meat Inspection Division, and the Food and Drug Administration. Progress has been made in coordinating the actions of these agencies and in communicating with industry.

Certain problems of communicating still remain in the area of Federal-State relations concerning the regulation of biologics. The Committee recommends that a paper or a panel discussion be presented at the next United States Livestock Sanitary Association meeting discussing the responsibilities of the states as cooperators with the Federal government in the regulation of both old and new biologics.

The Committee again endorses the recommendations made in the 1963 Committee Report relative to the use of pathogen free animals and classified cell lines for the production of biologics.

The Committee believes that it would be worthwhile to have a paper presented at the next United States Livestock Sanitary Association meeting describing the selection, usefulness and advantages of such animals and tissues in the production of biologics.

A record number of new biologics were introduced under special, limited and regular licenses during the past year. Several of these new
products have been questioned as to whether their use compromised in any way existing official control programs. One product was discontinued for this reason.

Contamination of several licensed products was found to exist and indicated that better self regulation by the industry and closer supervision by the government was needed.

Criticisms have reached the Committee concerning methods employed by experiment stations and universities engaged in the development of new biologics. The Committee hopes to examine some of these problems in more detail during the coming year so that a report containing recommendations as to ways and means in which industry, regulatory agencies, the scientific community and the professions may ultimately provide better biologics.
CONTROL OF ANAPLASMOSIS BY VACCINATION

C. C. Pearson

Pawhaska, Oklahoma

Since no completely effective treatment has been developed for anaplasmosis in its acute stages and present control methods are prophylactic antibiotic therapy and removal of carrier cattle after serologic identification. A vaccine which would prevent infection would be the ideal method of control.

With this ideal in mind, we have, during the past 18 years, tested many types of vaccine preparations, but were never able to prevent anaplasmosis infection. Since complete protection did not seem feasible in the near future, we have attempted to develop a vaccine which would provide sufficient resistance to prevent the appearance of the clinical disease. With this aim in mind, several antigenic preparations were tested for the degree of resistance they produced to the mortality and morbidity of anaplasmosis.

The antigen used in the vaccine for these experiments was prepared from anaplasma infected bovine blood by several steps to remove most of the plasma and cellular material. The remaining antigenic material was freeze-dried. Just prior to injection into cattle the desired amount of antigen was suspended in five ml of an adjuvant.

Two year old hereford cattle with a negative C-F test for anaplasmosis were used in the experiments as animals of this age usually exhibit obvious clinical disease with little mortality.

An estimation of resistance in vaccinated and unvaccinated animals was determined by the degree of anemia as measured by the packed cell volume (PCV) and the percentage of anaplasma infected erythrocytes appearing in the blood after challenge infection. Daily observations were made for evidence of illness or clinical symptoms of anaplasmosis. Each animal in the experiments was challenged with 0.10 ml of anaplasmosis carrier blood from a single sample of carrier blood.

In early experiments at this station the antigen was suspended in light mineral oil. Later other adjuvants have been developed or used, that are perhaps more efficient and easier to use. These are essentially emulsions of water and oil. A readily obtained fine homogenous suspension of the antigen in the emulsion is an advantage over the oil.

In previous work, reported at this meeting in part two years ago, two injections of the antigen 12 and 19 weeks apart were successfully used. In additional work to determine if shorter intervals were satisfactory it was found that six-week intervals between injections were equal in protection to the 12-week intervals. But at intervals of two weeks the protection or immunity obtained was not as effective in preventing anaplasmosis.

To determine the infectivity of the vaccine twenty doses of vaccine from three batches of vaccine were pooled making a total of 60 doses.
This was inoculated subcutaneously into a seplnectomized calf. At 12 and 26 days 750 ml of blood was taken and injected into splenectomized calves. All animals remained normal and no signs of infection could be determined.

Forty five cattle received two doses of vaccine at a six and twelve-week interval. Another ten were used as controls. They were challenged with 0.01 ml of carrier blood one to three months after the last dose of vaccine. One animal became slightly ill when the PCV declined to 16 percent. The other 44 cattle were equally divided between very slight anemia and no anemia. There were no clinical signs of anaplasmosis. The unvaccinated controls, all 10 head, developed anemia and clinical symptoms with a PCV mean of 11.8 (SD - 2.0), while the PCV mean of the vaccinated cattle was 27.8 (SD - 5.7).

In order to test the effectiveness of the vaccine when used under ranch conditions 200 grade Hereford cattle on a ranch in Osage County, Oklahoma were vaccinated. The cattle were in six pastures with 21 to 55 animals per pasture. In previous years five to eight percent of the cattle in this herd died each year. Prior to vaccination on May 1, the cattle were tested for anaplasmosis with the C-F test. Two doses of vaccine were given at six-week intervals. Carrier cattle were adjusted between pastures and additional ones added so that each pasture contained 10 percent carrier cattle.

Rather than depend on carrier cattle for challenge to the vaccine 12 additional cattle were infected with anaplasmosis and added to the herd, six in August and six in September. All of these became ill while in the vaccinated herd, and nine of the twelve cattle died of the disease.

All of the cattle were observed daily. Animals sick from any cause were reported and blood smears and PCV run. Only one animal became slightly sick from anaplasmosis.

One year after vaccination the number of carrier cattle in the herd was again determined by the C-F test. Previous work has indicated reaction to the C-F test due to vaccination disappears within one year after vaccination. Later work establishes this time as three to four and one half months. There was an overall increase of 10.5 percent C-F positive cattle in 1965 over 1964. This difference in carriers may be attributed to vaccinated cattle which became infected but did not show clinical signs of the disease.

There were no deaths during the year and when these results are compared with the history of the five to eight percent mortality per year due to anaplasmosis, there is indication of a relatively high degree of resistance to the clinical disease and death from anaplasmosis, conferred by the vaccine.

These experiments show the development of a vaccine which will increase the animals resistance and reduce the mortality of bovine anaplasmosis. Vaccinated cattle become carriers when challenged with anaplasma infected blood. Two doses of the vaccine are necessary, at not less than six-week intervals. The vaccine should be given subcutaneously, before or behind the shoulder.

Field trials of the vaccine under commercial ranching conditions resulted in a decrease in mortality from five to eight percent to zero percent.
The most important development in the field of anaplasmosis control during 1965 was the development, licensing and marketing of a vaccine for anaplasmosis. This is having an effect on many of the anaplasmosis programs that have been initiated. The vaccine brings to a culmination the research reported by Pearson, Kliewer and Brock of Oklahoma State University.1

The United States Department of Agriculture granted a biologics license for its manufacture and sale upon demonstration of its safety, usefulness, and the need for such a product. Recognizing that the efficacy of the vaccine is not yet fully known under all conceivable field situations in which its use may be indicated, the license is limited initially to two years while further research currently under way is evaluated. Also, the product is to be used only by a licensed veterinarian.

The vaccine is administered in two doses at least six weeks apart with a single annual dose thereafter. Satisfactory resistance is attained two weeks after the second dose. Local cattle handling practices can govern delay in administration of the second dose. Sanitary precautions must be taken in the form of separate sterile needles for each animal to avoid transmitting the disease from affected to susceptible individuals. It has been shown that the resistance derived by vaccinated animals under experimental challenge and field trial conditions, following the second injection, permitted susceptible animals to acquire anaplasmosis with few or no clinical signs of the disease. By preventing deaths from anaplasmosis and reducing clinical signs to a minimum, the deleterious economic effects of the disease can be greatly modified or eliminated.

The long-range effect of vaccine-produced resistance in affected herds is yet to be measured. Although the vaccine does not prevent infection under deliberate experimental challenge, there is a possibility that the spread of anaplasmosis within the affected herds may be slowed. However, no predictions can be made at this time as to whether or not this will be the case. The long-range persistence of effective levels of resistance in vaccinated individuals is also the subject of further study.

The vaccine stimulates production of complement fixing antibodies
which react with the antigens currently in use. The duration of reactions to the complement-fixation (C-F) test in the case of some of the early experimental vaccine was reported to be up to one year. Data now available to this Committee on the current vaccine, as marketed, indicate that the maximum duration of the vaccine-stimulated reaction is approximately four months.

The developmental experiments and field trials were carried out in cattle of the beef breeds. Trials are now under way to determine the efficacy of the vaccine in dairy breeds. It is expected that the protection levels will be comparable.

A survey as to which laboratories are conducting serologic tests for anaplasmosis has not been conducted during the past year. The 1964 report of this Committee is believed to closely approximate the current situation in this regard.

Chlortetracycline is permitted as an additive in the feed of beef cattle as an "aid in the elimination of the carrier state of anaplasmosis" under Federal regulations administered by the United States Commissioner of Food and Drugs within the limitations set forth. The Commissioner is now considering written objections, if any, to the intention published in the Federal Register of October 20, 1965, to include nonlactating dairy cattle under the same provisions. Objections must be filed with the Commissioner by November 13. Under the new provision, Item nine of Table 6 of paragraph 121.208(d), Part 121 (CFR), will read as follows:

"Principal ingredient - Chlortetracycline;
"Amount - five (milligrams per pound of body weight per day);
"Limitations - For beef cattle and nonlactating dairy cows; feed for 60 days; for use in the carrier state only; not to be fed within 10 days of slaughter; from a concentrate containing not less than two grams of chlortetracycline per pound. Labeling shall include a statement that a positive complement-fixation test at conclusion of a 60-day feeding period does not necessarily establish that anaplasmosis carrier state is still active. To positively establish that the carrier state has been eliminated, inject blood from a suspected carrier into a splenectomized (susceptible) calf;
"Indications for use - Aid in elimination of the carrier state of anaplasmosis."

Because the 60-day drug-feeding period exceeds the usual dry period practiced by dairymen, and because the date of parturition cannot be predicted precisely, the wording of the above limitations should be reconsidered. It is probable that the drug will sometimes be fed to animals which have initiated a new lactation period in order to complete the 60-day recommended feeding period. Because milk containing residues of chlortetracycline should not be consumed by humans, and because there are no contraindications for feeding chlortetracycline to lactating animals provided the milk is withheld from the public supply, the proposed statement of limitations set forth in Table 6 should be modified and so worded,
In relation to the five milligrams per pound of body weight feeding of chlortetracycline, current research indicates that the anaplasmosis carrier state can be eliminated by feeding lower levels (i.e., 0.5-1.0 mg. per pound body weight) of chlortetracycline over longer periods (i.e., 60-120 days) with fewer undesirable reactions. There are also indications that the CA test is a satisfactory indicator of the carrier state following the feeding of chlortetracycline.

Much research is under way in relation to anaplasmosis, particularly directed toward characterizing the causative agent. Such research should be continued and expanded. However, the knowledge of anaplasmosis now available, including diagnostic methods and procedures, exceeds that of many diseases which have been successfully eradicated in the past. Since the most desirable objective in disease control is to eliminate disease, rather than to continue to live with it, it is strongly recommended that eradication should continue to be the ultimate objective of the livestock industry in regard to anaplasmosis. In view of recent developments, it is the intention of this Committee to reconsider the Uniform Procedures for Anaplasmosis Control as recommended in the 1962 Proceedings of the United States Livestock Sanitary Association.

The Committee wishes to make the following recommendations:

1. That adequate funds be provided to carry out the following research:
   A. Study of anaplasmosis vectors and their control, particularly ticks.
   B. Study of anaplasmosis control on the basis of geographic areas.
   C. That research be continued to fully evaluate the vaccine now available and to find newer, more effective vaccines and preventatives.
   D. That research continue on the character, nature and life cycle of the causative agent.
   E. That anaplasmosis serology be expanded in all potentially productive areas, leading to new methods of detection and diagnosis,
   F. That research continue on the development of economical treatments for active and carrier states.

2. That any anaplasmosis immunizing agent developed which produces a serological reaction only be used officially under the authority of the Chief Livestock Sanitary Official of the respective State. However, it is the opinion of this Committee that the limitations on the marketing and use of the current vaccine are adequate. Therefore, this vaccine should be exempted from this recommendation.

REFERENCES
CERTIFIED BRUCELLOSIS-FREE STATUS: THE CONNECTICUT APPROACH

Philip D. Fichandler, D.V.M.*; Leander F. Williams, B.S., M.S.**; Jean V. Smith, D.V.M.***; Walter C. Ferrall, D.V.M.****

Connecticut was declared Certified Brucellosis-Free on January 15, 1965, the fifth state to attain this status. This achievement was due to the combined efforts and excellent cooperation of all groups in the livestock industry and the State and Federal agencies. The results of these efforts are presented.

In 1931 the control of infected animals, as determined by the tube agglutination test for brucellosis, was made the responsibility of the then existing Department of Domestic Animals (now the Livestock Division of the Connecticut Department of Agriculture and Natural Resources). The testing of the blood samples remained with the Department of Animal Diseases, College of Agriculture, University of Connecticut, where much of the early work on identification of the disease in cattle was done. As the program progressed, changes were made up-dating the procedures. Provision was made for the quarantine of reactors on the owner's premises, and herds that passed three negative tests over a 12-month period were certified free of brucellosis.

A State and Federal Cooperative (test and slaughter) plan was established in 1936, providing for the immediate slaughter of reactors and payment of indemnity. This plan also required that all imported cattle over six months of age must pass a negative blood test for brucellosis within 30 days of shipment. Informative blood tests and calfhood vaccination were made available to dairymen at state expense in 1941.

Vaccination of calves between the ages of four to eight months was made mandatory in 1945, except for calves in herds on the test and slaughter program. The use of desiccated vaccine was begun in 1946, after finding the liquid vaccine inferior due to a wide variation in the number of viable cells in different lots of vaccine. Four years later the age limit for vaccination was changed to six to eight months, following recommendations of the United States Bureau of Animal Industry (now the United States Department of Agriculture, Agricultural Research Service, Animal Health Division).

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****W. C. Ferrall is the Veterinarian in Charge, United States Department of Agriculture, Agricultural Research Service, Animal Health Division, Hartford, Connecticut.
Legislation passed in 1953 required that (1) each owner of bovine animals have all female calves (six to eight months old) vaccinated for the control of brucellosis, (2) no mature cattle shall be vaccinated with *Brucella abortus* vaccine, (3) imported cattle over 30 months of age must be negative to a standard tube agglutination test within 30 days before importation, and after entering this state and before they leave the premises of the importer, all such cattle shall be retested by a standard tube agglutination test approved by the Commissioner. Any such animals found to be positive shall be immediately slaughtered without indemnity, (4) after January 1, 1957, all imported female cattle over six months of age shall have been calfhood vaccinated and shall be from certified-free blood tested herds and, if over 30 months of age, shall meet the requirements in section (3), (5) a commission be established to make a study and a report on the eradication of brucellosis, said report shall include a recommendation of a date, not later than January 1, 1960, after which no milk may be offered for sale in Connecticut unless produced by brucellosis free animals.

In 1955, the Brucellosis Commission recommended that (1) after July 1, 1955, informative tests at state expense will be available to owners of livestock; (2) after April 1, 1956, all herds producing milk must be on an official blood testing program and tested within six months or be certified brucellosis free; (3) after April 1, 1957, no milk may be offered for sale unless from brucellosis free animals; all reactors must be immediately slaughtered and the premises cleaned and disinfected; (4) in all matters pertaining to the control and eradication of brucellosis in Connecticut there shall be no distinction made between cattle kept for dairying and for beef; (5) after July 1, 1955, testing of all cattle within an area shall be compulsory if the Commissioner finds that 75 percent or more of the cattle, or 75 percent of the owners of cattle within a given area have applied to place their cattle on an official testing program. Connecticut was declared a Modified Certified Area on July 26, 1957, the eighth state to attain this status.

In March 1962, a meeting of State and Federal officials was held and a date of July 1965 was set as a goal for certification of the entire state as brucellosis-free. In order to qualify for certification, as stated in Recommended Uniform Methods and Rules, there must be less than one percent of the herds and 0.2 percent of the cattle infected in an area during an 18-month period. The total number of herds and cattle under supervision in each county was tabulated and the allowable number of infected herds (less than one percent) and the allowable number of infected cattle (less than 0.2 percent) were computed (Table I). The infected herds were recorded with dates of disclosure of infection, and the start of the 18 months period was selected at a point that would permit the completion of required herd testing and allow for the possibility of newly infected herds being disclosed, while still remaining within the allowable percentage. For example, in one county the actual number of infected herds and the allowable number of infected herds were the same, with all the infected herds having been found in March 1962, so that April 1962 was used as the
<table>
<thead>
<tr>
<th>County</th>
<th>Herds Total</th>
<th>Allowable Infected</th>
<th>Actual Infected</th>
<th>Percent Infected</th>
<th>Cattle Total</th>
<th>Allowable Infected</th>
<th>Actual Infected</th>
<th>Percent Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairfield</td>
<td>470</td>
<td>4</td>
<td>4</td>
<td>.85</td>
<td>8,306</td>
<td>16</td>
<td>4</td>
<td>.08</td>
</tr>
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<td>Hartford</td>
<td>940</td>
<td>9</td>
<td>7</td>
<td>.75</td>
<td>21,536</td>
<td>43</td>
<td>21</td>
<td>.09</td>
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<tr>
<td>Litchfield</td>
<td>1,098</td>
<td>10</td>
<td>8</td>
<td>.73</td>
<td>34,045</td>
<td>78</td>
<td>10</td>
<td>.04</td>
</tr>
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<td>Middlesex</td>
<td>522</td>
<td>5</td>
<td>4</td>
<td>.80</td>
<td>7,160</td>
<td>14</td>
<td>4</td>
<td>.06</td>
</tr>
<tr>
<td>New Haven</td>
<td>671</td>
<td>6</td>
<td>5</td>
<td>.89</td>
<td>13,023</td>
<td>26</td>
<td>9</td>
<td>.06</td>
</tr>
<tr>
<td>New London</td>
<td>1,070</td>
<td>10</td>
<td>8</td>
<td>.75</td>
<td>23,043</td>
<td>56</td>
<td>15</td>
<td>.06</td>
</tr>
<tr>
<td>Tolland</td>
<td>690</td>
<td>6</td>
<td>3</td>
<td>.44</td>
<td>12,246</td>
<td>24</td>
<td>7</td>
<td>.05</td>
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<td>5</td>
<td>.50</td>
<td>17,840</td>
<td>35</td>
<td>6</td>
<td>.04</td>
</tr>
</tbody>
</table>
starting date for the 18 month period. In another county, the allowable number of infected herds was nine and the actual number of infected herds was seven, with the first of these being disclosed in June 1961. Using June 1961 as a starting date, if no more than two newly infected herds were disclosed, this county could qualify by January 1963. In this manner, a proposed target date was set for each county (Table II). The greatest difficulty in reaching certification was due to the number of infected herds rather than to the number of infected animals. In the majority of cases, only one or two animals in the infected herds gave a positive titer. The herds that were required to be blood tested were assigned for three months past the target date so as to permit an extension of the target date without requiring a large number of herds still to be tested.

Meetings were held with the County Agents, the Dairy Herd Improvement Association, the Extension Service, and representatives from the Diagnostic Laboratory, at which the proposed program and means of implementation were discussed. At county meetings, representatives from these agencies explained the program to herd owners and enlisted their support. Herd tests were assigned to private practitioners and ANH veterinarians contacted each practitioner to explain the program and to set a date two months in advance of the target date for completion of all testing. This was to allow for retests of any suspects or infected herds that might occur. A close supervision was maintained on the completion of assignments and in any herd where aid was needed an ANH veterinarian accompanied the practitioner. In herds where the owner was not cooperative or had improper facilities for testing, especially beef herds, the aid of the County Agent was enlisted and arrangements were usually completed quickly. ANH veterinarians performed the testing in herds where the practitioner was unable or unwilling to do so. The cooperation of the practitioners was outstanding, and in almost every case the assigned testing was completed by the date set, sometimes under adverse conditions. A friendly rivalry developed among County Agents and practitioners in an effort to become the first county certified.

As newly infected herds were disclosed, a continuing assessment of percentages of infected herds versus allowable herds was maintained and

<table>
<thead>
<tr>
<th>County</th>
<th>Earliest Possible Certification Date</th>
<th>Actual Date of Certification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairfield</td>
<td>Sept., 1963</td>
<td>Dec., 1964</td>
</tr>
<tr>
<td>Hartford</td>
<td>Dec., 1963</td>
<td>Dec., 1963</td>
</tr>
<tr>
<td>Litchfield</td>
<td>Jan., 1963</td>
<td>Dec., 1964</td>
</tr>
<tr>
<td>Middlesex</td>
<td>Sept., 1962</td>
<td>Nov., 1962</td>
</tr>
<tr>
<td>New Haven</td>
<td>Mar., 1963</td>
<td>Feb., 1964</td>
</tr>
<tr>
<td>Windham</td>
<td>Sept., 1962</td>
<td>Nov., 1962</td>
</tr>
</tbody>
</table>
where necessary, the target date was advanced as much as required to remain in the allowable tolerance. Disappointments became common, as in one county an infected herd was found on the last day, advancing the target date by three months. In other counties, more infected herds were found than were anticipated, requiring the date to be advanced as much as 15 months. A study of infected herds occurring during this period revealed that 76 percent of the reactors were either vaccinated or were suspected of being vaccinated beyond the recommended age. Persistant vaccinal titers in these animals were believed to have resulted in a reactor titer at adulthood, thus delaying certification of some counties for over a year.¹

In all cases of retests of animals disclosing a titer, an ANH veterinarian either accompanied the practitioner or performed the retest himself. A detailed epidemiological survey was performed on all animals with titers of less than 1:100 and in many cases owners of these animals were persuaded by ANH veterinarians, private practitioners, state officials, and County Agents to slaughter these animals. In infected herds, the status of the herd was determined by the use of a herd plot, incorporating any pertinent data such as vibriosis, mastitis, or leptospirosis. The herd history was obtained from state records of vaccination status, purchases and sales, and by a personal examination of the premises by an ANH veterinarian. A conference was then held with representatives of the various state and federal agencies involved, and a method of approach and recommendations for the possible solution of the problem in the herd were agreed upon and implemented. A continuing check of progress of the overall program was made by use of the Market Cattle Testing and Brucella Ring Test programs.

When a reactor was found a representative of the Livestock Division, Connecticut Department of Agriculture, visited the farm and appraised the animal (maximum: $275 for a grade animal, $325 for a purebred animal). The appraiser branded, tagged the animal and arranged for its immediate removal to slaughter and for the cleaning and disinfection of the premises under his supervision.

The laboratory procedure in the State of Connecticut for testing bovine blood samples for brucellosis is the standard tube agglutination test as outlined by the United States Department of Agriculture, Agricultural Research Service, Animal Health Division, Diagnostic Services.³

The Department of Animal Diseases, College of Agriculture of the University of Connecticut, is the only official testing laboratory in Connecticut. Blood sampling vials (13 x 100 m.m.) with cork stoppers are supplied by the laboratory to veterinarians authorized to make official brucellosis tests in Connecticut. The tubes are washed, fitted with new cork stoppers, and issued for re-use. Corks, when washed, may contain traces of serum in the pores so that it is more economical and satisfactory to use new corks. The tubes have an etched area for labeling.

The blood samples are either mailed to the laboratory or brought in by the veterinarian or by messenger. Tests on samples received at the laboratory before three P.M. are set up on the day of arrival, using an
individual pipette for each sample. Tubes containing the serum-antigen mixtures are incubated for 48 hours. A reading is then made using a constant light source supplied by two 15-watt fluorescent bulbs. Two laboratory technicians who have had several years experience in interpreting the tests make all of the readings. Tests are not set up on Saturday except under extenuating circumstances and with the approval of the supervisor of the laboratory. Tests set up on Thursday are read on Saturday. Reports from the laboratory are sent to the Livestock Division of the Connecticut Department of Agriculture and Natural Resources, which is the regulatory agency for the brucellosis program in Connecticut.

All blood samples that give a complete reaction in the 1:50 dilution or higher are tested by the standard plate test to verify the recording. A sample that gives a complete reaction in the 1:100 dilution is re-tested by the tube agglutination test, using serial dilutions to determine the titer of the serum. This procedure tends to eliminate human errors.

In herds that have a problem in achieving a negative status, a plot is made of the reactions of all the animals in the herd for the past several years, in an endeavor to find the problem animals. This information, in conjunction with vaccination data and epidemiological surveys, has been useful in identifying problem animals. When necessary, additional bacteriologic and serologic tests were made on milk and blood. In several herds, as soon as the "problem" animals were removed, it was possible to certify the herd within a short period.

The laboratory is staffed by two technicians who have received in-service training and a supervisor who is a qualified bacteriologist. The laboratory is capable of handling 700 samples a day, five days a week, with two technicians, or 168,000 samples per year. Under the present scheduling of tests, from 100 to 1,500 samples are received per day. Under these circumstances the laboratory tests between 60 and 70 thousand samples per year.

A reduction in the number of cases of brucellosis in man has paralleled the reduction of the incidence of infection in cattle in Connecticut. The Connecticut State Health Department reported 178 cases in humans in 1947, and the number has steadily declined each year until in 1963 and 1964 there were no reported cases in the state.

The effectiveness of this approach has been substantiated in the period that has elapsed since certification of individual counties. As of October 1, 1965, this period varies from 34 months in some counties to nine months in others (Table III).

On the basis of continuing blood tests of all cattle in these counties and quarterly Brucella Ring Tests of all dairy herds, only 16 herds in Connecticut had animals that were classified as reactors to the serum agglutination test during this period. In every case, only one animal in each herd was classified as a reactor, and upon removal from the herd no further reactors were found. Most of these reactors were either suspicious or a reactor to the tube test the first time they were tested in the herd. Five of these reactors were proved to be over-age at the time of vaccination with Strain 19 vaccine, eight were suspected of being over-age
<table>
<thead>
<tr>
<th>County</th>
<th>Date of Certification</th>
<th>Months Elapsed to Oct. 1, 1965</th>
<th>Reactors to Serum Agglutination Test</th>
<th>Herds with Suspicious Brucella Ring Test</th>
<th>Results of Blood Test of Suspicious Brucella Ring Test Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>New London</td>
<td>November 1962</td>
<td>34</td>
<td>2</td>
<td>13</td>
<td>4 suspects in 4 herds</td>
</tr>
<tr>
<td>Middlesex</td>
<td>November 1962</td>
<td>34</td>
<td>2</td>
<td>3</td>
<td>3 suspects in 2 herds</td>
</tr>
<tr>
<td>Windham</td>
<td>November 1962</td>
<td>34</td>
<td>3</td>
<td>6</td>
<td>Negative</td>
</tr>
<tr>
<td>Tolland</td>
<td>December 1962</td>
<td>33</td>
<td>2</td>
<td>6</td>
<td>2 suspects in 1 herd 1 reactor in 1 herd</td>
</tr>
<tr>
<td>Hartford</td>
<td>December 1963</td>
<td>21</td>
<td>3</td>
<td>8</td>
<td>2 suspects in 2 herds*</td>
</tr>
<tr>
<td>New Haven</td>
<td>February 1964</td>
<td>19</td>
<td>2</td>
<td>9</td>
<td>2 suspects in 2 herds</td>
</tr>
<tr>
<td>Fairfield</td>
<td>December 1964</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>Litchfield</td>
<td>December 1964</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>16</td>
<td>48</td>
<td>13 suspects in 11 herds 1 reactor in 1 herd</td>
</tr>
</tbody>
</table>

*One of these suspects later became a reactor

Note: Total herds in Connecticut: 6,331
Total cattle population: 137,199
at the time of vaccination from herd histories, two were purchased from out of state and were listed only as "official vaccinates," and one (titer 1:100) was from a small family herd, with no official record of vaccination.

During this period there were only 48 suspicious Brucella Ring Tests, and on the follow-up herd blood test 11 herds had suspects, and one herd had a reactor. This reactor was one of the two out-of-state purchases.

Market cattle testing revealed one reactor during this post-certification period.

REFERENCES

1. Fichandler, P. D.: "Man-Made Reactors" due to the Use of Strain 19 Vaccine. Manuscript to be published.
FURTHER STUDIES ON THE PERSISTENCE OF BRUCELLA ABORTUS STRAIN 19 IN BULLS

Ames, Iowa

Danks,¹ in 1943 reported the occurrence of orchitis and subsequent infertility in a bull previously vaccinated at six months of age with *Brucella abortus* Strain 19. Approximately one year postvaccination, an organism indistinguishable from Strain 19 was isolated from the epididymis of the right testicle.

More recently Lambert et al.,⁷ reported on the postvaccinal persistence of Strain 19 in two bulls. One bull vaccinated at six months of age developed a bilateral orchitis within 10 days. Two months postvaccination, bilateral castration was required, and Strain 19 was isolated from the testicles of this bull. The second bull was vaccinated at five months of age. Eight months later results of routine health tests disclosed the bull had a *Brucella* seroagglutination plate test titer of +50 and semen plasma test titer of +400. Strain 19 was isolated from each of three ejaculates of semen examined, although semen quality was not affected. At necropsy, 12 months postvaccination, Strain 19 was isolated from the seminal vesicles, prostate, urethra and the epididymides. Pathologic alterations were confined to the accessory genital organs.

An experiment reported here was designed to determine: (1) the frequency and nature of these sequelae to vaccination of bull calves with Strain 19 and (2) its potential significance in the brucellosis eradication program.

MATERIALS AND METHODS

Fifteen Holstein-Friesian bull calves were selected from the brucellosis-free herd at the National Animal Disease Laboratory. Throughout the entire experiment the bulls were confined to the isolation units at the laboratory.

Five bulls were vaccinated at exactly four months of age with Strain 19. Another group of five was vaccinated at exactly eight months of age. The remaining five bulls were vaccinated when exactly 10 months old. Viability counts of the vaccine* conducted on the day of vaccination were

From the Bacterial and Mycotic Disease Investigations, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Ames, Iowa.

The authors acknowledge the technical assistance of Richard C. Germer and Loren R. Elliott of this laboratory in the serologic and histopathologic examinations respectively.

*The vaccine was supplied through the courtesy of Diagnostic Reagents Section of the Diagnostic Services, Animal Disease Eradication Division, National Animal Disease Laboratory, Ames, Iowa.
from $1.3 \times 10^{10}$ to $1.6 \times 10^{10}$ *Brucella* organisms per ml.

Morning and evening rectal temperatures of each calf were recorded at eight AM and four PM on the day before vaccination and for 14 days after. The bulls were examined for evidence of local and systemic reactions for 21 days postvaccination.

**Blood examinations.**

(A) Serology: Blood collections from each calf consisted of one pre-vaccination sample obtained just before vaccination, daily samples for 10 days postvaccination, then samples on alternate days for three weeks, followed by weekly samples until 12 weeks and finally monthly samples until the termination of the experiment. All of the blood samples were tested for *Brucella* agglutinin content by the Standard *Brucella* seroagglutination tube test.\(^ \text{11} \)

(B) Bacteriology: Samples of blood were collected for bacteremia studies each time samples were obtained for serology. The methods employed for the demonstration of *Br. abortus* in the blood have been described previously.\(^ \text{9} \) In each instance where *Brucella* was isolated it was typed according to the presently recommended procedures for identification.\(^ \text{12} \)

**Examinations of the Semen.**

When the bulls attained the age of 12 months, samples of semen were collected employing either the artificial vagina or the electro-ejaculator\(^ * \) technique. Thirteen of the bulls responded to the artificial vagina technique, thus permitting collection of satisfactory or complete ejaculates from all of the 15 bulls. At least 10 complete ejaculates were collected from each bull at regular intervals between the ages of 12 and 18 months.

(A) Quality: Each ejaculate was examined immediately after collection for motility, morphology, color, concentration and percentage of live sperm.\(^ 2 \) A point scoring system was used, allowing a total maximum score of 100, with maximum points for each of the following categories being motility 40, morphology 30, gross appearance 20 and live/dead sperm ratio 10.

(B) Serology: Portions of each semen ejaculate were examined for the presence of *Brucella* agglutinins in the semen plasma. The antigen employed was that used in the *Brucella* standard seroagglutination tube test. The technique has been described previously.\(^ 10 \)

(C) Bacteriology: A 0.2-ml. amount of each ejaculate was serially spread on three plates of tryptose agar media enriched with seven percent bovine serum.\(^ 3 \) Duplicate series of plates of modified "W" antibiotic media were inoculated similarly.\(^ 4 \) Duplicate sets of both inoculated media were incubated aerobically and in the presence of an atmosphere containing 10 percent CO\(_2\). After five to seven days' incubation, the inoculated plates were examined for the presence of *Brucella*.

\( ^* \)Nicholson Manufacturing Co., Denver, Colorado.
Tissue examinations.—

When the bulls became 18 months old they were killed and subjected to bacteriologic and pathologic examinations.

(A) Bacteriology: The following organs were examined for Br. abortus using techniques described previously: liver, spleen, kidney, heart, lung, seminal vesicles, prostate, epididymides, testicles, bulbourethral glands, ampullae of the ductus deferens and pelvic urethra. The following lymph nodes were examined bacteriologically for Brucella: retropharyngeal, submaxillary, parotid, prescapular, bronchial, mediastinal, ileocecal, mesenteric, gastrohepatic, splenic, internal and external iliacs, deep inguinal, popliteal, and prefemoral. When glands were paired, both were examined.

(B) Pathology: The carcasses were examined for evidence of gross pathology, with emphasis on pathology of the reproductive tracts. Histopathologic examinations were performed on portions of the testicles, seminal vesicles, ampullae, bulbourethral glands, epididymides, prostate, and pelvic urethra. The tissues were fixed in 10 percent formalin, embedded in paraffin, sectioned at eight microns, and stained with hematoxylin and eosin. Approximately 25 sections from the genital tract of each bull were examined microscopically.

RESULTS

Postvaccinal increments above normal body temperature did not differ significantly among individuals or age groups. Generally the temperature rose from four to 4.5 F. above normal at approximately 24 hrs. postvaccination. The duration of an elevated body temperature varied from four to seven days.

Although size and persistence of local reactions at the site of vaccination varied considerably, there was no significant difference among bulls in the three age groups. Two bulls in each of the three age groups showed a pronounced systemic reaction after vaccination. Anorexia persisted until the third day in two bulls.

A mild transient orchitis, frequently bilateral, was detected in eight of the 15 bulls and was not confined to any one age group. The duration of the orchitis varied from two to 14 days, being most prominent from seven to 10 days postvaccination. The orchitis was characterized by heat, but there was little evidence of pain upon palpation of the testes.

Blood examinations.—

(A) Serology: The results of postvaccinal serologic studies are summarized (Table I). Maximal seroagglutinin titers were detected at 14 to 21 days' postvaccination. Generally titers persisted longer at diagnostic levels in bulls vaccinated at eight or 10 months of age than in bulls vaccinated when four months old. However, one bull (4-2) was an exception. The seroagglutinin titer of this four-month vaccinated bull had receded to 50 at four months postvaccination and remained at this level until the
### TABLE I
Results of Postvaccinal Bacteremic and Serologic Studies on Bulls Vaccinated at 4, 8, or 10 Months of Age with *Brucella abortus* Strain 19

<table>
<thead>
<tr>
<th>Group and Number of Bull</th>
<th>Persistence of Detectable Bacteremia (days)</th>
<th>Maximum STT Titer*</th>
<th>STT Titer When Bulls Were:</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>4-1</td>
<td>7</td>
<td>800</td>
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<td>4-2</td>
<td>28</td>
<td>400</td>
<td>200</td>
</tr>
<tr>
<td>4-3</td>
<td>22</td>
<td>800</td>
<td>50</td>
</tr>
<tr>
<td>4-4</td>
<td>5</td>
<td>800</td>
<td>25</td>
</tr>
<tr>
<td>4-5</td>
<td>6</td>
<td>800</td>
<td>25</td>
</tr>
<tr>
<td>8-1</td>
<td>0</td>
<td>3200</td>
<td>200</td>
</tr>
<tr>
<td>8-2</td>
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<tr>
<td>8-3</td>
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<td>1600</td>
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<td>8-4</td>
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<td>1600</td>
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</tr>
<tr>
<td>10-5</td>
<td>4</td>
<td>800</td>
<td>50</td>
</tr>
</tbody>
</table>

*STT = Standard Tube Test
Titer = Reciprocal of highest dilution in which agglutination was complete.

bull became 12 months old then the titer increased to 200. At necropsy (18 months of age) this bull's seroagglutinin titer was 100.

(B) Bacteriology: Postvaccinal bacteremia was demonstrated most frequently in bulls vaccinated at four months of age, with a total of 28 isolations from the five bulls (Table I). Bacteremia was demonstrable up to 28 days postvaccination in one bull (4-2) in this group.

Bacteremia was not detected at any time after vaccination in two of the five bulls vaccinated at eight months of age. Bacteremia could not be demonstrated in the remaining three bulls in this group later than 24 hrs. postvaccination.

Bacteremia was detected in all five bulls vaccinated at 10 months of age. Bacteremia was demonstrated up to 17 days postvaccination with a total of 16 isolations from these five bulls.

*Semen examinations.*—

The presence of *Brucella* agglutinins could not be demonstrated in any of the semen samples examined. The results of bacteriologic examinations of the semen for the presence of *Br. abortus* were consistently negative. All semen samples were normal in appearance, viability, morphology and motility. The majority of samples rated a score of 90 or higher (possible 100).
Tissue examinations.—

At necropsy all attempts to demonstrate the presence of *Brucella* in the various tissues were unsuccessful. There was no gross or microscopic pathologic alterations in any of the tissues examined.

DISCUSSION

Before the initiation of this experiment the authors had postulated that postvaccinal seroagglutinin titers persisted at diagnostic levels longer in bulls than in heifers. Data from a previous experiment\(^6\) was available for comparison of titers on heifers vaccinated at four or eight months of age. Our experimental data confirmed this hypothesis (Table II). At various times postvaccination, the mean seroagglutinin titers of five bulls vaccinated at four months of age are compared with titers of 24 heifers vaccinated at the same age. Similar data are presented on the five bulls and 23 heifers vaccinated at eight months of age. It is apparent from the table that titers of bulls vaccinated at eight months of age persist at substantially higher levels than titers of bulls vaccinated at four months of age. Titers of bulls vaccinated at four months of age, persist at approximately the same level as heifers vaccinated at eight months of age. Titers that persist in bulls of breeding age frequently confuse interpretation of test results and hinder the progress of brucellosis eradication.

**TABLE II**

Comparative Persistence of Postvaccinal Seroagglutinin Titers of Bulls and Heifers

<table>
<thead>
<tr>
<th>Postvaccinal Time (months)</th>
<th>Mean Postvaccinal Seroagglutinin Titer</th>
<th>4 months vaccination</th>
<th>8 months vaccination</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>3</td>
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<td>110</td>
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<tr>
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</tr>
<tr>
<td>12</td>
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<td>40</td>
<td>8</td>
</tr>
</tbody>
</table>

*Mean titer at 10 months postvaccination.

Early research conducted on large numbers of cattle, shortly after the discovery of Strain 19, failed to show any evidence of localization or persistence of the organism after calfhood vaccination.\(^8\) The authors have received verbal reports of infertility in bulls allegedly due to vaccination with Strain 19. However, evidence was always circumstantial, and confirmatory bacteriologic and histopathologic examinations were lacking. Although semen from our bulls was not used in breeding experiments there was no reason to suspect that impaired fertility existed, as indicated by semen morphologic examinations.
Under our experimental conditions, the frequency of persistence of Strain 19 in bulls appears to be less than one in 15. To what extent the uniform and restrictive environmental conditions in the isolation units at the laboratory contributed to the results is unknown. One may speculate on what the results might have been if the bulls had been permitted a more farm-like or natural environment. Nevertheless, the previous reports cannot be ignored that Strain 19 actually has persisted in the reproductive tract and semen of three bulls up to 12 months. This potential danger in itself should favor the discontinuance of the practice of vaccinating bulls.

SUMMARY

Fifteen bulls from four to 10 months old were vaccinated against brucellosis in an experiment designed to determine the nature and frequency of persistence of *Brucella abortus* Strain 19. The bulls were confined to an isolation unit throughout the experiment. A mild transient postvaccinal orchitis was detected in eight bulls. Postvaccinal bacteremia was detected in 13 of the 15 bulls and persisted up to 28 days in one bull. Postvaccinal seraagglutinin titers persisted longer at diagnostic levels in bulls than in heifers vaccinated at the same age in a previous experiment.

Semen samples collected from the bulls had no noticeable decrease in quality and were consistently negative to the *Brucella* semen plasma test. *Brucella* was not isolated from any of the semen samples. The bulls were killed at 18 months of age and at necropsy there was no evidence of gross or microscopic pathologic alterations. Strain 19 could not be demonstrated in any of the tissues.

REFERENCES


The past year has provided great encouragement to brucellosis eradication but has also caused concern that progress in some areas has slowed to near stagnation. The encouragement is derived from the excellent progress being made in modified certified areas attaining Certified Brucellosis-Free status. Among the noncertified States, however, with the exception of one, only eight and one-half percent of the remaining non-certified counties achieved modified certified status during the last year.

This severe slowing may, however, be the impetus to move these areas to recognize the need to hasten certification. Leaders among the livestock producers in several States have already taken action to accelerate eradication in their States through various means such as obtaining increased appropriations, more effective laws and regulations and

**BRUCELLOSIS ERADICATION**

**BLOOD TESTING: CATTLE**

![Graph](image)

Figure 1

*Chief Staff Veterinarian, Brucellosis Eradication, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.*
greater cooperation of the entire livestock industry.

There were approximately 11,500,000 cattle tested during fiscal year 1965. This includes farm and ranch testing and animals tested in the market cattle testing program (Figure 1). There were 129,199 reactors disclosed. More than two out of every three reactors came from non-certified States (Figure 2). This is not because of greater activity in seeking the infected animals since most of the certified States do have effective surveillance procedures underway. It is just another expression of the high incidence of brucellosis in some of the noncertified areas. Several States have reported an incidence greater than seven percent infection in the herds and lots tested. Active brucellosis eradication work is underway in only certain counties of the noncertified States, leaving most of the noncertified counties an untested unknown area as far as brucellosis is concerned. The noncertified States (Figure 3) contained over 55 percent of the herds found infected in the Nation during the past year. When it is recognized that animals move from herds of unknown status in these non-area counties on the basis of a single test, the hazard of spreading infection becomes readily apparent. Eradication work must be intensified in the noncertified areas to lessen the threat of reinfection to those areas already certified.

![BRUCELLOSIS ERADICATION](image)

**REACTORS FOUND**
- IN 36 STATES P.R., & V.I (Certified as of June 30, 1964)
- IN 14 STATES (Not certified as of June 30, 1964)

Figure 2
CERTIFICATION

Only one State, North Dakota, achieved modified certified status during fiscal year 1965. Two States, Arkansas and Indiana, lost modified certified status, but regained that status during the period. Florida and Alaska were the only States which did not achieve certification of a single county. Most progress was made in Iowa where 41 of the 84 counties to achieve modified certified status during the year were located. As of September 30, 1965, there were 2,256 modified certified counties and 30 modified certified States in the Nation (Figure 4).

While the number of new modified certified counties was alarmingly low, the activity in qualifying new areas free was encouraging. The States of Connecticut, Wisconsin and Vermont achieved Certified Brucellosis-Free status. One hundred seventy-two counties were certified-free, making a total of 470 counties Certified Brucellosis-Free in 25 States, Puerto Rico and the Virgin Islands. Currently, seven States and the Virgin Islands are Certified Brucellosis-Free in their entirety. The certified-free areas contain 329,000 herds and over eight million cattle.

The combined certified-free areas as of September 30, 1965, including both modified and free, total 2,734 counties or over 87 percent of all of the counties in the Nation (Figure 5). With the inclusion of the 134 counties conducting area work at that time, it can be stated that over 90 percent of the counties are actively working to complete the eradication of brucellosis.
Brucellosis Eradication Program
CERTIFIED AREAS
September 30, 1965

COUNTY CERTIFICATION STATUS
Cooperative State-Federal Brucellosis Eradication Program

Figure 4

Figure 5
BRUCELLOSIS RING TEST

The number of herds marketing milk continues to decrease; however, the number of cows per dairy herd increased at approximately the rate necessary to absorb the decrease resulting from these dairy herd dispersals. As the incidence of the disease decreases in dairy herds, most States are increasing the number of BRT collections. During Fiscal Year 1965, 17 States were conducting the test four times per year; 29 States three times per year; and four States are still unchanged from two times per year. The increased BRT rate has a compound benefit. The infected herd is found earlier and the danger of spread to other herds is lessened by early location of the infection. In this fiscal year a 0.9 percent of the herds tested were BRT suspicious (Figure 6). This is unchanged from the previous year.

The increasing amount of milk and number of cows per herd has made necessary studies to determine methods to adjust the sensitivity of the ring test as a means of locating infected animals in the large herds. It would appear that this can be accomplished by adjusting the volume of milk used in the test. Complete information will be published as soon as the results of this study are available. The reappearance of dichromate compounds in the Babcock sample preservative led to a study which
indicates that use of the commercial tablet containing the dichromate causes a decrease in the BRT titers to the extent that positives may become negative.

The brucellosis ring test is without question one of the major factors responsible for the rapid progress in brucellosis eradication in many areas. The fact that it is so efficient and economical to conduct makes it imperative that the ring test be applied to the fullest extent possible. Continuing studies must be made to insure that these qualities are maintained as milk marketing practices change.

MARKET CATTLE TESTING

Market Cattle Testing was incorporated in the brucellosis eradication program in 1959. It has made encouraging growth during the year (Figure 7). Fiscal year 1965 is the first year in which there was a greater number of samples collected at slaughter than from live animals at markets. There was a 110 percent increase in the number of animals backtagged over the last fiscal year. There was a continuing rise in the percent return of brucellosis samples from backtagged cattle. During the six-month period from January to June 1965, the rate of return was 55 percent. The success of market cattle testing is dependent upon full cooperation of all the States in tag application and also in increasing the efficiency of blood sample collection. An outstanding example is Wisconsin

**MARKET CATTLE TESTING PROGRAM**

_Cows Blood Tested_

<table>
<thead>
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<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows Blood Tested</td>
<td>803,912</td>
<td>1,833,962</td>
<td>2,999,020</td>
<td>3,750,452</td>
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</tr>
</tbody>
</table>

**Brucellosis Eradication**
Brucellosis Eradication

WISCONSIN BLOOD TESTED 93,000 BACKTAGGED COWS FROM 28 STATES

Figure 8

collecting and testing more than 93,000 blood samples from cattle originating in 28 other States (Figure 8). This may appear on the surface merely to be a lot of work for the Wisconsin laboratory, but it is to their advantage to hasten the eradication of brucellosis throughout the Nation, lessening the chance of exposure of cattle from outside sources. Epidemiological studies indicate that several severe outbreaks of the disease in the State of Wisconsin were the result of importation of breeding cattle from noncertified areas.

Combining market cattle testing from all sources, 3-3/4 million animals were tested, disclosing 50,667 reactors. As a result of these MCT reactors, 11,429 herds were tested, 2,063 of which were found to have 12,636 additional reactors. These infected herds of origin of MCT reactors were found to have a greater than nine percent incidence rate. Full use is not yet being made of MCT results. Several of the noncertified States lack the manpower to test the herds of origin of MCT reactors located in non-area counties. With the existing manpower shortage, consideration should be given throughout these areas to make full use of MCT for initial certification. This would eliminate a tremendous amount of testing of non-infected herds since even in an area of heavy infection it is unusual to find more than 10 percent herd infection.
There were 6,832,394 calves vaccinated for brucellosis during the fiscal year. This is approximately 400,000 less than the previous year. High level calf vaccination has been urged for many years in those areas of high incidence of disease. The response has been less than encouraging. Some of these areas vaccinate no more than 50 percent of their retained heifer calves. Other areas of the country where the infection rate has been low have never used vaccine in any appreciable amount, and no increase has been seen in the incidence of the disease.

It has been mentioned in previous years that vaccine has advantages and limitations. In order that eradication can be achieved as early as possible, vaccine should be used to its full advantage and in such a manner that its limitations are minimized. For example, in high incidence areas vaccination of as near 100 percent as possible of all heifer calves to be retained for breeding purposes at the earliest possible time after they have attained four months of age is desirable. In the areas of low incidence, vaccination becomes an expensive and unnecessary practice which can be detrimental to prompt eradication of the disease from herds which may become infected. The fact remains that it is not uncommon to find an infected vaccinated animal carrying only a suspicious blood test, remaining in the herd and exposing other animals in the herd to brucellosis. Studies are currently underway to determine the feasibility of reducing the vaccination age in order that residual titers resulting from vaccination be lessened.

During the past year additional States have recognized that they are approaching the point at which the need for vaccine is negligible. New York, Vermont and Wyoming have repealed their laws and regulations requiring vaccination of animals imported into the State.

**BRUCELLOSIS PROBLEM HERD PROGRAM**

The brucellosis problem herd program is one of the most dramatic accomplishments of the eradication program. It has demonstrated to the owner that an infected herd can be freed of the disease. When a herd that has been infected for years is cleared up in a few months of intensive work with a brucellosis epidemiologist, that farmer or rancher is convinced that brucellosis can be eradicated.

A brucellosis problem herd program is now being conducted in 27 States. The basis for this activity is the especially trained brucellosis epidemiologist who has capabilities for interpreting supplemental test results and when indicated, conducts bacteriological studies to determine the presence of *Brucella* organisms in animals not indicated as infected by standard procedures. The infected animal carrying a blood serum agglutination titer less than a reactor is still a common factor in causing persistence of infection within the herd.

A two-week course was held at the University of Minnesota in which men were trained for work in four States. Currently, six veterinarians
have completed graduate study leading to a master's degree and are assigned to field work in strategic areas making them available for consultation with the epidemiologists having had less training. There are an additional six veterinarians engaged in postgraduate study in brucellosis epidemiology at this time. Very few of the certified States do not have a problem herd. In order that brucellosis can be promptly eradicated, it is urged that each of the certified States have an especially trained epidemiologist to work in the infected herds in that State.

Over and above the benefits derived from the eradication of brucellosis from problem herds, these men are making a valuable contribution to the total knowledge of the disease as it occurs in the field.

SWINE BRUCELLOSIS

Progress in the eradication of brucellosis from swine has moved rapidly during the year. Two States, Vermont and Utah, completed testing of all the breeding swine within the State. On June 30, California had 42 validated counties, Nevada two, Hawaii and Georgia each had one, making a total of 89 counties validated brucellosis-free (Figure 9).

Dooly County, Georgia, the first county validated three years ago, was revalidated by testing all of the breeding swine. The card test was used on this occasion, and the entire county was tested in 11 days compared to approximately six weeks the first time Dooly County was tested.

States with Validated Brucellosis-Free Swine Herds

![Figure 9](image-url)
Major emphasis in the swine brucellosis eradication program is the establishment of validated brucellosis-free herds especially among the purebred breeders, building a large source of brucellosis-free swine from which both commercial and purebred breeders can obtain breeding animals for establishing and enlarging their herds. Iowa, California, Hawaii and Indiana have the greatest number of validated brucellosis-free herds which on June 30 totaled 2,208.

SUMMARY

In summary it should be recognized that adequate methods are currently available to eradicate brucellosis. Nevertheless, efforts are continuing to find more efficient, and wherever possible easier methods for the livestock producer.

Cooperative studies with several of the States in improving methods for locating infected herds and animals have been conducted during the year. Examples are: BRT studies in large dairy herds in California, backtagging and improved blood collection methods at slaughter in several midwestern States and use of a modified antigen and card test in range herds. With cooperation of the livestock industry, there should be no problem in achieving the goal of complete eradication of swine brucellosis by 1975.
CALFHOOD VACCINATION: ITS RELATION TO BRUCELLOSIS ERADICATION

H. G. Wixom, D.V.M.* and L. C. Vanderwagen, D.V.M.*

In a report to this Association in 1944, Dr. A. W. Miller, then Chief of the Bureau of Animal Industry, made the following statement regarding brucellosis eradication. "We know now, as we have known since the first years of our control program, that we cannot hope to succeed by the use of any one method, but that success must depend on the use of all facilities, with probable stress on one or more. It is evident that under the present conditions stress must be placed on vaccination."

The brucellosis program has made tremendous progress in the 21 years since Doctor Miller made his report. We have made full use of all our facilities, and special stress has been placed on the calfhood vaccination program. It has become the backbone of our eradication effort. Vaccination is practiced in all 50 states, with 45 states having incorporated it into the official program for more than 10 years. The United States Department of Agriculture progress report shows that 55 percent of all eligible heifers in the nation were vaccinated last year. A recent survey conducted by our Department reveals that 20 states are vaccinating nearly 100 percent of all dairy animals and 12 states report that vaccination is compulsory.

The conditions of 20 and 30 years ago that Doctor Miller referred to are no longer present. At that time, brucellosis was rampant throughout the cattle population. The disease caused storms of abortions to occur, resulting in extreme financial hardship to many. Losses to the industry were estimated to approach $100 million annually. The morbidity rate within infected herds was extremely high, as evidenced by the records which show that 11.5 percent of the blood tests conducted in 1935 were positive. To further illustrate this high infection rate, consider for a moment that 29 percent of the cattle in the 240 herds used to evaluate Strain 19 vaccine in the late 1930's under field conditions were reactors. The only tools available and being used at that time were test, slaughter, cleaning and disinfection, segregation and quarantine. The newly developed vaccine offered hope in that it promised to reduce this high infection rate through a highly resistant cattle population which would not be susceptible to the ravages of the disease that were so costly to the industry. We note how this hope has now been fulfilled.

RESULTS OF VACCINATION

Compare the conditions of the 1930's and 1940's to the conditions of today. Where once we had hundreds of herds that had successfully

*Division of Animal Industry, California Department of Agriculture, Sacramento, California.
eliminated the disease, we now find hundreds of counties, and even entire states, that are certified brucellosis free. Last year 1.2 percent of animals tested were reactors.

The calfhood vaccination program must be given credit for its role in this progress. Reports in abundance reveal that in well-vaccinated herds the infection rate is reduced nine and tenfold. At the 63rd annual meeting of this body, Dr. J. E. Stuart, et al., presented California's experience with 11 years of vaccination. Their report showed that well-vaccinated herds had only one-tenth the number of reactors that nonvaccinated herds were disclosing. In 1959, the United States Department of Agriculture reported a survey of Montana beef cattle revealed the infection rate within the vaccinated cattle population to be approximately 80 percent lower than in the nonvaccinated population. State after state reported similar findings as vaccination practices became widespread.

In California, surveys indicated 16-17 percent brucellosis infection in our dairy herds and seven to nine percent infection in our beef herds prior to initiating a statewide calf vaccination program in 1948. We initiated a blood testing program nine years later. Although the herd infection rate was high, the reactor rate was shown to have been reduced to an apparent two percent in dairy cattle and 0.7 percent in beef cattle. During the period from 1957, when we began our testing program, to August, 1962, when our State became a modified certified area only 24,000 reactors had been removed from our herds. Let me contrast this with another dairy state where, in 1952, alone there were over 151,000 reactors removed and still that state had not achieved modified certified status.

The powers of Strain 19 are not surprising nor unique. The effectiveness of vaccines in reducing disease is well known to members of this Association.

VACCINATION CREATES SOME PROBLEMS

There is, however, the other side of the coin to consider showing that vaccine can create some problems.

Strain 19 vaccine contains viable organisms and, as such, presents the inherent problems found in this type of vaccine. These problems are being mentioned more and more by the officials of those states that are in the final stages of eradication. In the recent nationwide survey conducted by our Department 31 states anticipate a certified brucellosis free status within the next five years. The majority of the officials in these states report that the drawbacks and problems caused by Strain 19 vaccine are becoming more and more evident as eradication of brucellosis is approached.

Paramount among the problems expressed by these officials is the so-called persistent vaccine titer. The existence of these titers was recognized early in the program. From the 1953 Proceedings of the National Brucellosis Committee we read as follows: "In the fiscal year 1938-39, the percentage of suspects in Wisconsin cattle was 2.67 percent. This was before calfhood vaccination was allowed in this state. In the
fiscal year 1950-51, after calfhood vaccination was well advanced, there were 7.41 percent suspects. In the first three months of the fiscal year 1952-53, there were 16.1 percent suspects." The allowance of a "vaccine titer tolerance" was a realistic approach to this problem, and reduced the number of animals classed as suspects. But the titers remained, even though their classification was changed.

We, in California, currently find nine suspects for every reactor in our well-vaccinated cattle population. Additionally, there are usually three cows with some titer for every suspect. Thus, for each reactor disclosed, we find 25 or more cattle with demonstrable titers using the blood test dilution of our present official program. This means that 17 percent of the animals blood tested reveal what could be defined as danger signals.

These titers by sheer numbers rule out any attempt by the owner to segregate or isolate animals until their disease status is determined. Yet they cannot be ignored, for they may mask infection, thus permitting a reservoir of infection to remain in the herd. On several occasions during the past two years, our laboratories have cultured viable strains of *Brucella abortus* from this class of animal. We, therefore, retest all suspects at 30-day intervals, which adds a considerable work load. More than 100 lots containing up to 600 of these suspects were retested each month last year with more than 15 percent of these lots disclosing infected animals.

A reduction of the vaccination age to four months would, no doubt, aid materially in reducing the number of persistent titers to an extremely low level. At this time in California, we are conducting an educational program aimed toward reducing the vaccination ages of both dairy and beef calves. It will be several years, however, before the resulting reduction in titers will become evident.

Vaccination also presents another drawback to the testing phase of the program. Heifers under the 30 months of age limit may have been exposed and become infected, but they move freely into the main herd or from one herd to another without test. The recent lowering of this 30-months age limit helped materially in this area, but has not completely solved the problem. In order to overcome infection in some herds, we have had to test all of the calves and young heifers in a herd, despite the fact that they have been vaccinated, in order to detect the animals that may be harboring real infection.

Another problem becoming evident from our epidemiological studies of infected herds in certified free counties, is the fact that on many occasions we isolate an organism from reactor animals that is indistinguishable from Strain 19. This opens up an entirely new field; one that should be thoroughly investigated. This finding may be significant in the continued use of vaccine, particularly in areas of low incidence of the disease.

A final problem, although not mentioned by any of the state officials surveyed is the sense of security felt by the livestock owner who vaccinates. After California went on a statewide vaccination program
abortions due to brucellosis practically vanished. As we know vaccination lowers or eliminates clinical evidence of the disease from the herd, thus the livestock owner may not be concerned or, in some instances, may be antagonistic regarding eradication efforts.

BRUCELLOSIS PROGRAM ADVANCES

The brucellosis program is moving through a series of methodical, ever-advancing steps toward final eradication, incorporating all useful and effective procedures, with stress on one or more at each step. The first step or objective was to reduce the incidence of the disease in the herd and protect the owner from the ravages that were ever present. Test, slaughter, and segregation procedures were part of this step, but special emphasis was placed on calfhood vaccination. The second step, removing the infected animals from the herd, placed special stress on the testing of all cattle on an area basis. Here, vaccination continued, but importance was placed on slaughter, quarantine, and cleaning and disinfection procedures.

The third step, tight surveillance to locate and remove the remaining reservoirs of infection, will soon be here for each of us, if it is not already here. In this step we must continue to make full use of all of our tried and true facilities, with special emphasis on these tools that are most effective in locating and identifying these reservoirs. We now have many more tools to help us. The screening programs, Brucellosis Ring Test (BRT) and Market Cattle Testing (MCT) have been developed and expanded with this thought in mind. To be effective, it is important that these screening programs be used extensively with fast reporting and efficient traceback. All evidence of potential infection must be thoroughly investigated. Epidemiological procedures, which include the supplemental blood and milk tests, are essential aids in tracking down and identifying infected animals that might be missed under routine testing conditions. Personnel who are skilled in the use and interpretation of these tests as well as in all phases of epidemiology, are fast becoming available. These procedures are expensive and time consuming, but pay tremendous dividends.

VACCINATION IN OUR CURRENT SITUATION

What part does vaccination play in our present phases of eradication? When we moved from just controlling the disease to an effort to eradicate it, we were in a sense saying that if eradication can be a reality then the time will come when vaccination as well as routine blood testing and screen testing, would not be necessary. Eradication must be kept as the goal before us.

Further, rather large State and Federal appropriations have been made to accomplish the objectives of the program. Under this arrangement we are required to show progress, and I believe we have excellent progress to show for our efforts; however, we must bear in mind that the
appropriations will not be continued forever. In the future, the appropri-
ations for brucellosis eradication may be limited and officials may need
to evaluate where the appropriated funds should be used to be most ef-
fective in accomplishing the objective of complete eradication.

GUIDELINES

In those states where the eradication program is well advanced and
the officials have good reason to believe that vaccination may be inter-
fering with the further progress of eradication it may be desirable to de-
emphasize vaccination in favor of achieving complete eradication. Before
this is done in any state we believe certain guidelines should be con-
sidered. Two of these guidelines we have already indicated:

First Guideline. How significant are the actual problems created by
vaccination within the State, and

Second Guideline. Utilization of available funds to accomplish the
most gain.

A third guideline should be the phase that a state or area may be in.
Obviously vaccination should not even be considered to be eliminated as
long as the area is in the initial phases of the program. Under these
phases, vaccination is the backbone of the program. Our experience in
California has been that, even though we have been a modified certified
state for three years, vaccination has not really interfered with eradica-
tion efforts, but has actually strengthened our efforts in some problem
herd situations. If we had eliminated vaccination in these herds, I am
sure that we would have faced some serious disease-spreading problems
and, in addition, some public relation problems. In many cases, we have
solved the disease problems by a combination of efforts—placing the herd
on a consistent routine vaccination program and applying all the testing
techniques available. In some sections of the Nation, the eradication pro-
gram may have advanced so far that the benefits derived are outweighed
by the problems created and, in such cases, emphasis on vaccination
would have to be evaluated. We have not reached such a point yet in
California.

A fourth guideline is the relative size of herds and the number of im-
ports. These two items perhaps should be considered together. In Cali-
fornia, our herds are large and our cows are expensive. Though we are
Number five in the number of dairy cows in the Nation, we are fourth in
total milk produced, and third in the cash receipts from milk and cream.
California dairy cows lead the Nation in production of both milk and butter
fat, averaging 10,810 pounds of milk in 1964. This is 37 percent above
the national average in milk. Place this type of animal in herds ranging
in numbers up to 2,000 milking cows, and you have some different prob-
lems as compared to herds small in number and average in value. Fur-
thermore, we import 2,830,000 cattle plus 1,590,000 swine per year.
Many of these animals come from areas not yet modified certified.
Though many of these imports go for slaughter or to feed yards a large
number may be introduced into our herds or be in contact with them.
Also we have considerable movement of animals within the State. To eliminate vaccination under such conditions would be dangerous, as indicated from our past experience, where we could not eliminate brucellosis from our large herds without the aid of vaccination.

It has been expressed that, when the incidence of infection is low, our screening devices may permit us to locate the infection and quickly overcome it. There is no doubt that the screen tests will detect infected herds. But the importance of the time lag between introduction of the disease into the herd and detection by the screen test cannot be ignored. In some instances this time lag may be several months. In our well-vaccinated dairy herds, only a few reactors may be revealed following a suspicious BRT, but in nonvaccinated highly susceptible dairy herds a much higher reactor rate could be found. It is conceivable in such susceptible herds that most of the herd could become infected before the disease is detected.

The BRT is also affected by the dilution factor in our large herds. A BRT sample from the entire herd can represent several hundred or even several thousand animals. If the test is negative the ranch must be visited, and individual lots of cows sampled. Otherwise early outbreaks would go undetected. This slows down our BRT sampling procedure. As to the MCT program, the cull cattle sampled under this program move to market under seasonal conditions, and it may be some time between tests from an individual herd. Foci of infection could be introduced and the disease become well established in susceptible cattle before the disease was found. Last year our MCT reactor herds revealed 10 reactors per herd on initial herd test.

I do not wish to imply that the screening tests are not doing an important job in the eradication effort, but we should look at the problem squarely and realize that these screening procedures may have some limitations. In some states these limitations may not be acute nor important, but they are in our State. Therefore, depending on the relative size of herds and the number of imports into herds, as well as where these imports are from, should be considered as a guide.

Some concern has been expressed by this Association regarding those states that require vaccination on cattle entering that state. In California, we have such a requirement. Our concern is not to keep the exporting state on a vaccination program, but provide protection to those animals that may be introduced into our herds having a residual of infection. Many of our dairy herds replace 30-50 percent of these animals annually many of which are imports. This could mean that within two to three years the entire herd could be unprotected. Since adult vaccination is not feasible our California industry feels that they must continue to require vaccination of imports until they are confident that the disease has been eradicated within our State. This will mean that the exporting state will have to continue some vaccinations to market their animals in California. This should not be a serious problem if vaccination is done at the lower ages.

A fifth guideline is what controls are to be established on the vaccine. There has been mentioned phasing out of vaccination, but I have heard little mention made of what controls should be exercised regarding
vaccine. Many states apparently have no control of brucella vaccine, and indiscriminate use could be a more serious problem than controlled official vaccination. As in the hog cholera eradication program, control of the vaccine is an important factor if the ultimate goal of eradication is to be achieved.

My sixth and final guideline is industry cooperation. In the individual herd approach to disease control, the cooperation of the owner is assured, but in an area eradication effort, the cooperation of the industry is vital to the success of the program. Recently a leading cattleman commented to a federal official, when phasing out of calf vaccination was mentioned: "Doctor, you are frightening us." I have a great deal of respect for this cattleman because of his progressive outlook on disease control and eradication work and because of his support to our brucellosis program in years past.

One state official mentioned to me that he thought I was even courageous to mention the words "phasing out" because of the concern that had been expressed to him by his livestock industry.

I have received numerous personal contacts and letters from industry people on this subject. The general expression was that vaccination should be continued until eradication has been completed. A leading veterinarian commented to me not long ago, "What next, now I understand that they are planning to eliminate calf vaccination? How far out can we get?" I mention these to illustrate some of the deep feelings regarding vaccination.

When the testing phase of eradication was approached, many industry members accepted the testing program because they had difficulty in moving their animals interstate, or were finding difficulty in marketing their dairy products. From their standpoint, however, they were not particularly troubled with brucellosis, as vaccination had overcome their major problem. They supported the program to overcome their movement or marketing problems, but the individual benefits, as far as the disease was concerned, were not clear. Now, there is the rumor among them that vaccination may be eliminated, and they are concerned.

Furthermore, a change is being considered by this Association to reduce or eliminate a serological tolerance for vaccination. To many in the industry, this change is tied to the elimination of vaccination; though the recommendation is specifically aimed at removing infection from herds. This has already caused alarm among the industry in some states, and questions are being presented as to the need for this change. Our experience has shown that, when infection is established, even the low-titered animals may be infected. There is definitely a need for flexibility in handling infected herds in order that possibly infected animals may be removed. This, however, has been made possible through the use of supplemental tests applied by the specially trained epidemiologists. To apply this proposed change across the board may result in elimination of a needless number of noninfected animals. Furthermore, considerable resistance may be manifested by the industry unless they understand, or flexibility is provided.
We must have industry support as we advance in our program. Information on the changes ahead and the reasons for the changes must precede the regulatory phase. At this point, however, many of our industry people, as well as the members of our profession, are not convinced that elimination of vaccination prior to complete eradication is feasible. One leading industry member presented this question: "Has this phasing out of vaccination ever been tried?" The experiences of the Scandinavian countries have been cited as an answer, but these are countries far away and animal husbandry in these countries is not fully understood by our industry. Thus the answer is not convincing. Yet the real answer to this question is the key to unlocking some of the problems we will face in the future.

CONCLUSION

The calfhood vaccination program has been and continues to be an important part of our eradication effort. In some states vaccination is more important than in others. In some states vaccination may actually be interfering with eradication. Each state needs to analyze its own situation.

In accordance with the guidelines we offer, there may be states or even parts of states that can and should move to determine the safety of eliminating certain procedures and provide confidence to us all for the future steps that may need to be taken.

SUMMARY

Vaccination has proven to be the decisive factor in the effort to eradicate brucellosis, particularly in large herds and in areas importing replacement animals. Its use continues to be essential for control and eradication of brucellosis in many areas of the country. However, vaccination does create some problems in the eradication effort. In some States, vaccination may be interfering with routine blood and screen testing procedures designed to detect and positively identify the last reservoirs of infection. Eradication is our goal and this implies that a time will come when vaccination and routine testing will not be necessary. The eradication program is advancing. In order to move from where we are to where we want to go may require some changes. Guidelines are offered for consideration by the various states as they face these changes.
REPORT OF THE COMMITTEE ON BRUCELLOSIS

A. O. Wilson, Hysham, Montana, Chairman; J. V. Smith, Hartford, Connecticut; J. R. Bishop, Atlanta, Indiana; J. S. Brenner, Grant, Montana; J. B. Finley, Encinal, Texas; D. E. Flagg, Bismark, North Dakota; A. E. Janawicz, Montpelier, Vermont; W. D. Knox, Fort Atkinson, Wisconsin; R. Laramore, Gillette, Wyoming; C. A. Manthei, Ames, Iowa; J. L. McAuliff, Courtland, New York; S. H. McNutt, Madison, Wisconsin; W. A. Moynihan, Ottawa, Canada; C. K. Mingle, Hyattsville, Maryland; W. C. Tobin, Denver, Colorado; H. G. Wixom, Sacramento, California

It gives me such pleasure to present to you the report of the Brucellosis Committee. On Tuesday, October 26th, an opening meeting was held and the attendance was very gratifying. Tremendous interest was shown by industry regarding various phases in the development of our eradication program. The facts presented by those in attendance have been very helpful to the Committee in preparing this report. We sincerely trust that this interest will continue and with your advice and recommendations our goal of brucellosis eradication will be attained at an early date.

Since our last meeting, 71 counties have been declared Modified Certified and 180 counties have qualified as Certified Brucellosis-Free Areas. Also, one State achieved a Modified Certified status and three States attained a Certified Brucellosis-Free status. At this time, 37 States have achieved the Modified Certified status. Of these, seven States and the Virgin Islands have gone on to reach the ultimate goal—a Brucellosis-Free status.

SEROLOGICAL AND AGE TOLERANCES FOR OFFICIAL VACCINATES

Last year the Committee considered a reduction or elimination of titre tolerances on official vaccinates effective January 1, 1967. However, the evidence presented in this Committee by Dr. Paul Nicoletti, Dr. Joe Hendricks and Dr. E. A. Schilf of the Animal Health Division of the United States Department of Agriculture indicated that a change at this time was not desirable. More work is to be done and when evidence is presented the Committee will give the proposal further consideration.

The age tolerance was discussed at length. Evidence was presented to the Committee that it would be advantageous to the eradication program to reduce the age of testing of officially vaccinated animals of the dairy breeds to 20 months and beef animals to 24 months.

RECOMMENDED AGES FOR VACCINATION

At the last meeting of the Committee it was recommended that all calves be vaccinated as near four months of age as possible. At this
time we want to reemphasize this program. It was also stated that re-
search would be conducted to determine if vaccination at ages earlier than
four months would provide servicable immunity. This work is now in
progress and we are now waiting until results are available.

DISCONTINUING MARKET CATTLE TESTING OF DAIRY COWS

Although the milk ring test now provides reasonably effective cover-
age for the Nation's dairy herds, there is an urgent need for further ex-
pansion of surveillance testing of beef herds. The Committee believes
that funds employed for blood testing dairy cows under the market cattle
testing program could be used to better advantage in expanding market
cattle testing of beef cows. Therefore, it is recommended that testing of
dairy cows for brucellosis in the market cattle testing program be dis-
continued and that funds saved by this action be used for expanding the
surveillance testing of non-milking herds.

It is further recommended that identification of dairy cattle be con-
tinued as a means of assisting the tuberculosis eradication program.

MILK PRESERVATIVES AND THE BRUCELLOSIS RING TEST

All States should be alerted to the possibility of milk samples being
preserved by certain products, recently introduced, which can cause a
positive Brucellosis Ring Test to be recorded negative. We ask the
United States Department of Agriculture to keep all State officials ad-
vised of products which may affect BRT readings.

RECERTIFICATION

It is the recommendation of the Committee that the period of time for
the blood testing of individual herds not adequately screened be extended
from 18 months to 24 months for purpose of recertification.

It has also been recommended that consideration be given to the de-
velopment of procedures that would provide for the continuous certifica-
tion of areas where adequate screen tests are being maintained thereby
eliminating anniversary dates.

FEDERAL FUNDS

Federal funds available for support of the cooperative State-Federal
brucellosis eradication program continue to be inadequate to meet re-
quests from all the cooperating States. In view of the importance of pro-
viding maximum support possible to those States that are not yet certi-
fied and are conducting effective programs, the Committee recommends
that the United States Department of Agriculture critically evaluates ex-
isting eradication programs in the non-certified States and give serious
consideration to transferring support from the ineffective State pro-
grams to those States where effective programs need additional support.
MARKET CATTLE TESTING

The Committee has reviewed and endorses the following recommendation adopted by the National Brucellosis Committee at the February 1965 annual meeting in St. Paul, Minnesota:

Whereas, market cattle testing provides a valuable means of locating infected animals in non-lactating herds; and

Whereas, the market cattle testing program provides continuous surveillance of large numbers of herds at relatively low costs; and

Whereas, market cattle testing alleviates the need for on-ranch testing of negative herds;

Therefore, it is recommended that an increased effort be made to utilize the market cattle testing procedure to screen all eligible animals moving through marketing channels not already screened by the milk ring test.

Be it further recommended that the United States Department of Agriculture review with the officials of the States concerned with the United States Department of Agriculture costs of vaccination in an effort to divert funds to an expanding Market Cattle Testing program for beef cattle.

THE BRUCELLOSIS CARD TEST

A report presented to your Committee indicates that the card test for cattle has merit as a screening test, particularly in range areas. We suggest the use of the test be limited to its screening function until an evaluation has been made by the Sub-committee on Research of the National Brucellosis Committee and its findings and recommendations made to the 1966 annual meeting of the United States Livestock Sanitary Association.

We commend the swine industry for its increasing use of the card test and urge its further expanded use.

SWINE BRUCELLOSIS

The Committee reviewed its previous recommendations on methods for the eradication of swine brucellosis and found them good.

Available information suggests that brucella infection in swine is gradually becoming less and less. This can be attributed to a number of factors, but especially it is the result of a better understanding of the infection and a desire on the part of producers not to live with so dangerous a disease—a realization that one dare not risk exposure to such evil infection. Still, the decrease in swine brucellosis is not progressing as rapidly as the decrease in bovine brucellosis. As bovine brucellosis eradication nears completion, demands for more rapid eradication of swine brucellosis will multiply. In this instance swine producers may face unnecessarily harsh restrictions. This would be unfortunate because swine brucellosis is readily eradicated and could be accomplished now.
REPORT OF COMMITTEE

FREEDOM IN MOVEMENT OF CATTLE

This Committee in the past has taken the stand that there should be more freedom of movement of cattle originating in Certified Brucellosis Areas. We would like to reaffirm this stand and recommend at this time that all States that have Modified Certified Areas do all they can to make the movement of these cattle as unrestricted as possible.

INTER AREA MOVEMENT OF CATTLE

In last year's report, the Committee expressed its concern about the hazards associated with movements of animals from non-certified areas and urged those States that were not yet certified to increase their efforts in this direction. At that time, there was a total of 484 non-certified counties in 11 States. Currently, there are still 398 counties that have not yet qualified as Modified Certified Areas. In view of these continuing delays and the urgent need for protecting the vast majority (87 percent) of the Nation's counties that are well advanced in their efforts to eradicate brucellosis, this Committee recommends 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 Brucellosis Areas or Certified Brucellosis-Free Areas. It is further recommended that each State adopt similar regulations controlling intrastate movement of cattle.

SUPPORT OF MARKET CATTLE TESTING

The Market Cattle Testing procedure is one of the most efficient methods of implementing and maintaining a constant surveillance in disease control particularly regarding burcellosis and tuberculosis.

The Committee wishes to commend the packers, meat inspectors, and market people for their untiring and dedicated efforts which have produced great accomplishments in the area of brucellosis eradication.
LEPTOSPIROSIS IN CALIFORNIA DAIRY CATTLE
A SEVEN YEAR STUDY

Robert E. Carroll, D.V.M.

Historically *Leptospira pomona* has been considered to be the principal etiologic agent for bovine leptospirosis in the United States.\(^1\) Recent reports from various sections of the country indicate the growing awareness toward other serotypes in the etiology of this disease complex.\(^2,3,11,12,13,15,16,18\) Serologic findings combined with bacteriologic confirmation would appear to indicate a need for revising serologic testing procedures to include a battery of at least 12 leptospiral serotypes, together with a re-evaluation of vaccination procedures for controlling this disease.\(^4\)

This report describes the various serotypes of leptospires associated with clinical disease and the use of polyvalent bacterins in California dairy herds. The evaluation of the bacterins is based on clinical observations of milk production and reproduction efficiency under field conditions.

MATERIALS AND METHODS

Approximately 50 dairy herds composed of 10,000 cows and heifers of milking age represented in this study are located in Orange and Los Angeles counties. Data has also been accumulated from herds in Riverside, San Bernardino and Kings counties during the last two years. All herds participated in a veterinary health management program. Dairying is conducted on a dry lot basis with very little pasture utilization. Artificial insemination with veterinary supervision is commonplace and replacements primarily are purchased from counties in the San Joaquin valley area and such states as Idaho, Utah, Nevada, Montana, and Wisconsin. Dairy management is designed to achieve the ultimate in herd unit productivity and reproductive capacities. The progressive attitude toward dairy management made this study possible. Herd efficiency can be gauged by the inter-related factors of milk productive capacity and herd reproductive status. Milk production values are a good indicator of herd health and herd management as disturbance in animal health has immediate repercussions upon all phases of dairy management and production averages. The common denominator among herds in this study is the manifestation of leptospirosis. One such herd (10) is represented in Table I. Two years of production averages for herd 10 are evaluated on the basis of DHIA testing. Table I represents monthly figures based upon fat averages per productive unit. The year 1958 is selected as the base year depicting the true productive capacity of the herd unit. The first indications of a leptospira problem were seen in June 1960 with subsequent amplification in 1961. The year 1962, depicted in Table II, represents the loss of productive efficiency due to the leptospira problem.
TABLE I
Two years of Production Averages (Fat per Productive Unit)
Depicted by Line Graph for Herd 10

<table>
<thead>
<tr>
<th>Months of year</th>
<th>Average Pounds Fat Per Productive Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>F</td>
</tr>
<tr>
<td>M</td>
<td>A</td>
</tr>
<tr>
<td>J</td>
<td>A</td>
</tr>
<tr>
<td>S</td>
<td>O</td>
</tr>
<tr>
<td>N</td>
<td>D</td>
</tr>
</tbody>
</table>

Year: 1958
Lbs. Milk: 2,964,512
Lbs. Fat: 107,132
Salvage: 76
Replacement: 68
Cow Inventory: 12-31-58=181

Year: 1969
Lbs. Milk: 3,362,121
Lbs. Fat: 120,993
Salvage: 71
Replacement: 96
Cow Inventory: 12-31-59=206

Year: 1970
Lbs. Milk: 3,458,493
Lbs. Fat: 119,112
Salvage: 107
Replacement: 107
Cow Inventory: 12-31-60=206

Year: 1971
Lbs. Milk: 3,394,549
Lbs. Fat: 119,077
Salvage: 101
Replacement: 94
Cow Inventory: 12-31-61=199

Year: 1972
Lbs. Milk: 3,262,190
Lbs. Fat: 112,760
Salvage: 118
Replacement: 147
Cow Inventory: 12-31-62=228

In Table II an attempt is made to depict a more accurate interpretation of the economic manifestations of the leptospira problem in Herd 10. Production recorded by the creamery, in terms of fluid milk and fat, is represented in the light of replacement demands necessary to obtain such production. In the year 1962, 375 animals, representing 180 milking units in any given month, were required to obtain production levels presented as compared to 249 animals in the year 1958. The loss of monies in lowered production in 1962 was in reality doubled by replacement costs, approximately 65 percent of herd 10 was replaced during that year.
Serological Survey.—Serums were examined for the presence of leptospiral agglutinins by the macroscopic slide agglutination procedure and/or the agglutination-lysis methods. Early in the study three test antigens, *L. pomona*, *L. canicola* and *L. icterohemorrhagiae* were used routinely and later expanded to include 12 test antigens.\textsuperscript{8,9}

Sera were taken from symptomatic cattle and for survey purposes a 10 percent sampling of asymptomatic animals were conducted. Within the past year microscopic-agglutination tests have been conducted by the Leptospira Reference Laboratory, WRAIR, upon sera submitted from our laboratory. All sera (256) were examined for agglutinins against 21 leptospiral antigens.\textsuperscript{10}

Isolation procedures.—Attempts to recover leptospires from affected cattle from the inception of this study in 1958 were unproductive until 1962 at which time the first of three isolates was made. Whole blood and voided undiluted urine were utilized for culture in artificial media and for animal inoculation.\textsuperscript{6,18} Subcultures of all leptospiral isolates were sent to the Veterinary Division, WRAIR, or CDC, USPHS for identification.

### RESULTS

**Serological Survey.**—Results of the macroscopic slide agglutination test are shown in Table III. The four predominant serotypes detected were *L. grippotyphosa*, *L. australis*, *L. pyrogenes* and *L. canicola*. A herd designated herd 42, involving 10 leptospiral antigens using the microscopic-agglutination test is presented in Table IV. A significant reaction

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Total # Animals Tested</th>
<th>Total # (+)</th>
<th>% +*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. grippotyphosa</em></td>
<td>185</td>
<td>113</td>
<td>61**</td>
</tr>
<tr>
<td><em>L. australis</em></td>
<td>172</td>
<td>102</td>
<td>60</td>
</tr>
<tr>
<td><em>L. pyrogenes</em></td>
<td>172</td>
<td>91</td>
<td>53</td>
</tr>
<tr>
<td><em>L. canicola</em></td>
<td>371</td>
<td>184</td>
<td>50</td>
</tr>
<tr>
<td><em>L. pomona</em></td>
<td>398</td>
<td>170</td>
<td>44</td>
</tr>
<tr>
<td><em>L. sejroe</em></td>
<td>332</td>
<td>132</td>
<td>40</td>
</tr>
<tr>
<td><em>L. ballum</em></td>
<td>171</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td><em>L. autumnalis</em></td>
<td>359</td>
<td>128</td>
<td>36</td>
</tr>
<tr>
<td><em>L. mini-georgia</em></td>
<td>319</td>
<td>111</td>
<td>35</td>
</tr>
<tr>
<td><em>L. icterohemorrhagiae</em></td>
<td>389</td>
<td>128</td>
<td>33</td>
</tr>
<tr>
<td><em>L. bataviae</em></td>
<td>172</td>
<td>46</td>
<td>27</td>
</tr>
<tr>
<td><em>L. hyos</em></td>
<td>320</td>
<td>67</td>
<td>21</td>
</tr>
</tbody>
</table>

*Agglutination of 1+ through 4+.
**Percentages given are to the nearest whole number.

### TABLE III

Examination of Bovine Serum Samples from Suspected Animals by Means of Macroscopic Slide Agglutination Procedures with 12 Leptospiral Serotypes: 1958 through 1962
<table>
<thead>
<tr>
<th>Serotype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>% Positive* (≥1:100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. canticola</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>L. icterohaemorrhagiae</td>
<td>1600**</td>
<td>1600</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>1600</td>
<td>1600</td>
<td>58</td>
</tr>
<tr>
<td>L. grippotyphosa</td>
<td>100</td>
<td>1600</td>
<td>1600</td>
<td>100</td>
<td>1600</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>1600</td>
<td>1600</td>
<td>100</td>
</tr>
<tr>
<td>L. pomona</td>
<td>1600</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>1600</td>
<td>1600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>&quot;R&quot; (Calif.)</td>
<td>1600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>L. patoc</td>
<td>100</td>
<td>1600</td>
<td>400</td>
<td>400</td>
<td>1600</td>
<td>1600</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>L. wolffi</td>
<td>1600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>L. autumnalis</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>L. australis</td>
<td>1600</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>L. hyos</td>
<td>1600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42</td>
</tr>
</tbody>
</table>

*Percentages given to nearest whole number.

**Reciprocal of highest dilution.
has been considered at 1:100, with predominant serotypes manifested as L. grippotyphosa, L. icterohemorrhagiae, L. patoc, L. autumnalis, L. pomona and L. australis. The presence of L. patoc as a screening antigen shall be discussed later. A member unit of this herd, agonal at time of sampling, yielded a mixed culture of L. grippotyphosa and L. icterohemorrhagiae. Early in 1965, 62 sera taken from member units of five leptospirosis positive herds were submitted to the Leptospira Reference Laboratory, WRAIR (Table V). Predominant serotypes were L. borinicana, L. wolffi, L. pomona, "R" (Calif), and L. canicola. In April of this year an additional 194 sera from two herds with signs of leptospirosis were examined for leptospiral agglutinins by means of the microscopic-agglutination test (Table VI). The predominant serotypes in this series were L. cynopteri, "R" (Calif), L. wolffi, L. borinicana, L. pomona and L. hyos. Titers of 1:1600 or greater were not evident.

Results of isolation attempts.—During the seven year period the many attempts which were made to isolate leptospires were unsuccessful. The first of three isolates was made from herd 10 in 1963. Whole blood and undiluted voided urine from an abortion cow five months in gestation were used to inoculate a guinea pig and the resulting isolate was identified as L. canicola by the CDC, USPHS.

A second culture identified by the Leptospirosis Reference Laboratory, WRAIR as a mixed L. icterohemorrhagiae and L. grippotyphosa culture was isolated from a cow of herd 42. The isolation was made by means of both direct and indirect culture. The animal yielding the culture was symptomatic at the time of sampling. Table IV presents a titration of symptomatic sera at approximate time of isolation. Serological

<table>
<thead>
<tr>
<th>TABLE V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titration Results on 62 Bovine Serum Samples (Microscopic-Agglutination) to 19 Leptospiral Serotypes 1965. Herds 5, 40, 15, 50 and 17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Dilutions 1:100</th>
<th>Dilutions 1:400</th>
<th>Signif. %* + (≥1:100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. canicola</td>
<td>5</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>L. borinicana</td>
<td>11</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>L. icterohemorrhagiae AB</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>L. wolffi</td>
<td>10</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>L. icterohemorrhagiae AG</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&quot;R&quot; (Calif)</td>
<td>6</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>L. hyos</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>L. butembo</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>L. pomona</td>
<td>8</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>L. patoc</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>L. balaur</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>L. autumnalis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other**</td>
<td>8</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

*Percentages given to the nearest whole number.

## TABLE VI

Titration Results on 194 Bovine Serum Samples (Microscopic-Agglutination) to 19 Leptospiral Serotypes 1965. Herds 5 and 40

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Dilutions 1:100</th>
<th>Dilutions 1:400</th>
<th>Dilutions 1:1600</th>
<th>Signif. %* + (≥ 1:100)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. cymopteri</em></td>
<td>94</td>
<td>20</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>'R' (Calif)</td>
<td>100</td>
<td>12</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td><em>L. wolfii</em></td>
<td>39</td>
<td>10</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td><em>L. autumnalis</em></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>L. butembo</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>L. pomona</em></td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td><em>L. bornicana</em></td>
<td>33</td>
<td>2</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td><em>L. ballum</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L. icterohemorrhagiae AG</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>.5</td>
</tr>
<tr>
<td><em>L. hyos</em></td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><em>L. canicola</em></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>L. grippotyphosa AI</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>.5</td>
</tr>
<tr>
<td>Other**</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Percentages given to the nearest whole number.
**Includes *L. batauiae*, *L. pyrogenes*, *L. australis*, *L. javanica*, *L. djasmann*, *L. alexi*, and *L. celebboni*.

detection of leptospiral agglutinins in sera obtained from guinea pigs utilized in the isolation procedures, gave presumptive evidence 30 days post-inoculation of a mixed infection. In the battery of test antigens utilized by serological survey techniques, both *L. icterohemorrhagiae AG* and *L. grippotyphosa AI* are noted.

The third isolate was obtained from an agonal cow in herd 5. The affected animal was anemic, cachetic, relatively icteric and purpuric. Whole blood and urine were used for both direct and indirect culturing. The cow died 36 hours later. A member of the hebdomadis serogroup was suspected serologically. Serological titration of sera from guinea pigs, 30 days postinoculation, indicated presumptive evidence for *L. australis* infection. More complete testing has indicated a close relationship between the isolate and the *L. figlexa* types particularly strain "patoc." It is for this reason that "patoc" was included in the antigen battery for a segment of the serological survey Tables IV and V). Identification studies are still in progress.

**Multiserotype Vaccine Efficacy.**—Multiplicity of leptospiral infections suggested a need for a polyvalent or multiserotype vaccinal approach to leptospira control.

The first attempt at multiserotype vaccination was in 1962, utilizing the commercially available *L. pomona* bacterin in conjunction with a bivalent canine biologic containing inactivated whole culture lysates of *L. canicola* and *L. icterohemorrhagiae*. The venture was inconclusive and not economically feasible. At this time a bovine polyvalent leptospiral vaccine was received from a middlewestern manufacturer for clinical field evaluation work and referred to as "PCI," containing *L. pomona*, *L.
TABLE VII
Effect of Vaccination with Polyvalent Leptospira Bacterin on Total Production in Herd 10

<table>
<thead>
<tr>
<th>Month</th>
<th>Pre-infection (Normal) Production Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>9,800 - 9,600</td>
</tr>
<tr>
<td>Aug.</td>
<td>9,600 - 9,400</td>
</tr>
<tr>
<td>Sept.</td>
<td>9,400 - 9,200</td>
</tr>
<tr>
<td>Oct.</td>
<td>9,200 - 9,000</td>
</tr>
<tr>
<td>Nov.</td>
<td>9,000 - 8,800</td>
</tr>
<tr>
<td>Dec.</td>
<td>8,800 - 8,600</td>
</tr>
<tr>
<td>Jan.</td>
<td>8,600 - 8,400</td>
</tr>
<tr>
<td>1962</td>
<td>8,400 - 8,200</td>
</tr>
<tr>
<td>1963</td>
<td>8,200 - 8,000</td>
</tr>
</tbody>
</table>

"PCI" Booster

The leptospira problem reached its maximum intensity during July 1962. In Table VII the effect of "PCI" bacterin upon herd production is graphically illustrated. Production rose from 8193 lbs. to 9021 lbs. within 11 days of vaccination. A "booster" vaccination was administered with production at 9010 lbs. and approximately 45 days later production assumed proportions of 10,001 lbs. fluid milk. It must be stated that during the period of July 1962 to January 1963, no changes were made in dairy management, care or feeding. In addition, weather conditions remained dry and milk with no demonstrable variations during this period. No replacement cows were purchased during the months of November 1962 through February 1963. The incidence of fresh cows was minimal, by comparison with previous years and the period of summer and early fall 1962. This latter feature, again a reflection of the immensity to the leptospirosis problem.

Remission of the leptospira problem, in all of its manifestations, was accomplished for a period of approximately five months with subsequent recurrence. Severity, at this time, was appreciably less due to recognition and ability to employ relative vaccinal control. Duration of remission has been established at approximately five to six months with this biologic in all herds represented in the study. Productive efficiency, as
established by baseline years, has returned in all herds vaccinated with "PCI" bacterin or its successor "Lepto 5," and with much the same intensity of significance as in herd 10. The inclusion of *L. hardjo* and *L. grippotyphosa* to the tri-valent bacterin and therefore referred to as "Lepto 5," was an effort to more accurately satisfy serological requirements.

Excellence in dairy management is dependent upon the interrelated factors of reproduction and milk production. In the chronically affected leptospira herd a loss of reproductive efficiency anticipates lowered production capacities. In herd 10, the period from February 1, 1962 to October 1962, immediately preceding vaccinal leptospiral control had a reproductive status of 2.2 services per conception, based upon confirmation of pregnancy and a 13.3 month calving interval. Following vaccination, during the period from November 1962 to June 1, 1963 the herd had a reproductive status of 1.8 services per conception, based upon confirmed pregnancy and a 12.0 month calving interval for 154 animals processed. Nineteen herds have been selected for demonstration of reproductive efficiency during a two year period (1963 to 1965) (Table VIII). Eighteen of the nineteen herds, excluding herd 41, are considered leptospiral problem herds. These herds were selected for demonstration as all were vaccinated with the polyvalent leptospiral bacterin (Lepto 5) in September or October 1963 and 1964 with a subsequent "booster" vaccination in October or November 1963 and 1964. The attempt has been to "booster" vaccinate within 45 days of initial vaccination. It is noted that the period extending from July to October 1964 presents the peak disturbance in reproductive status for herds presented. This was also found to be true in 1963. This period of ninety days would seem to represent the peak in the cyclic phenomena of leptospira morbidity in other herds represented in this study. A gradual return of reproductive efficiency is noted following this period and following vaccination. Herd 41 is presented to illustrate that the apparent loss of productive efficiency is due to an increase in leptospira morbidity in affected herds and not due to inclement weather condition or the like.

**DISCUSSION**

Due to the complexity and multiplicity of bovine leptospiral infections, it is apparent that a very real need exists for polyvalent multiseroype leptospiral vaccines. The very nature of the disease in a given dairy cow population presents a diagnostic challenge to the clinician. Chronic and insidious in nature with an undulating morbidity curve, leptospirosis is often lost in the course of differential diagnosis. Although eradication may be impossible, control is highly probable. Serologic diagnosis must include the use of multiple antigens preferably by means of microscopic-agglutination test. This study indicates the fallacy of restricting the diagnosis of bovine leptospirosis to a single serotype *L. pomona*.

Bacteriologic confirmation of the disease by isolation of *L. canicola, 'R' (Calif)* and mixed *L. icterohemorrhagiae and L. grippotyphosa* culture
TABLE VIII
Reproductive Status of 19 Herds for Period from June 1963 to July 1965

<table>
<thead>
<tr>
<th>Herd No.</th>
<th>6-63-1-64 S/C</th>
<th>6-63-1-64 #Cows</th>
<th>1-64-7-64 S/C</th>
<th>1-64-7-64 #Cows</th>
<th>7-64-10-64 S/C</th>
<th>7-64-10-64 #Cows</th>
<th>10-64-1-65 S/C</th>
<th>10-64-1-65 #Cows</th>
<th>1-65-4-65 S/C</th>
<th>1-65-4-65 #Cows</th>
<th>4-65-7-65 S/C</th>
<th>4-65-7-65 #Cows</th>
<th>Range of calving interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>1.50</td>
<td>135</td>
<td>1.50</td>
<td>78</td>
<td>1.86</td>
<td>52</td>
<td>1.75</td>
<td>77</td>
<td>1.50</td>
<td>36</td>
<td>1.50</td>
<td>42</td>
<td>11.6-12.9</td>
</tr>
<tr>
<td>18</td>
<td>2.10</td>
<td>146</td>
<td>1.60</td>
<td>128</td>
<td>2.16</td>
<td>67</td>
<td>2.09</td>
<td>119</td>
<td>1.97</td>
<td>107</td>
<td>1.52</td>
<td>32</td>
<td>12.0-14.3</td>
</tr>
<tr>
<td>36</td>
<td>1.70</td>
<td>200</td>
<td>1.85</td>
<td>161</td>
<td>2.08</td>
<td>71</td>
<td>1.91</td>
<td>125</td>
<td>1.73</td>
<td>84</td>
<td>1.75</td>
<td>98</td>
<td>12.1-12.7</td>
</tr>
<tr>
<td>25</td>
<td>1.71</td>
<td>174</td>
<td>1.68</td>
<td>229</td>
<td>1.82</td>
<td>82</td>
<td>1.66</td>
<td>113</td>
<td>1.73</td>
<td>102</td>
<td>1.71</td>
<td>101</td>
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</tr>
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<td>29</td>
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<td>1.76</td>
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<td>125</td>
<td>2.04</td>
<td>172</td>
<td>1.71</td>
<td>151</td>
<td>1.70</td>
<td>104</td>
<td>12.0-13.4</td>
</tr>
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<td>2.40</td>
<td>200</td>
<td>1.70</td>
<td>150</td>
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<td>133</td>
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<td>130</td>
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<td>76</td>
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<td>2.32</td>
<td>116</td>
<td>2.28</td>
<td>205</td>
<td>2.15</td>
<td>159</td>
<td>1.68</td>
<td>120</td>
<td>11.7-13.4</td>
</tr>
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<td>487</td>
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<td>32</td>
<td>1.87</td>
<td>73</td>
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<td>1.87</td>
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<td>1.65</td>
<td>152</td>
<td>1.58</td>
<td>131</td>
<td>1.69</td>
<td>183</td>
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<td>1.54</td>
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<td>75</td>
<td>2.55</td>
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<td>113</td>
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<td>99</td>
<td>1.95</td>
<td>92</td>
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<tr>
<td>Av.</td>
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<td>4028</td>
<td>1.74</td>
<td>3458</td>
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<td>1768</td>
<td>1.94</td>
<td>2456</td>
<td>1.82</td>
<td>1842</td>
<td>1.68</td>
<td>1592</td>
<td>12.2-12.5</td>
</tr>
</tbody>
</table>

*Services per conception, based upon confirmation of pregnancy, expressed to nearest decimal.
ROBERT E. CARROLL

demonstrates multiplicity to leptospiral infections. To the author's knowledge the culture referred to as mixed \textit{L. icterohemorrhagiae} and \textit{L. grippotyphosa} reported here represents the first such isolation from a dairy cow.

Demonstration of efficacy of any biologic presents inherent problems. The criterion of success in dairy management is the ability to attain a maximum level of reproductive efficiency and its subsequent maintenance and attain maximum levels of unit production. It is to this end that a measurement of efficacy was attempted. By no means does the author infer that complete control has been attained, as no control animals were available.

In this era of veterinary health management and the establishment of a closer liason between the profession and dairy management, the control of such an important economic disease as leptospirosis is of paramount importance.

SUMMARY

Data has been presented in the form of serological evidence and bacteriologic confirmation to substantiate the need for a polyvalent vaccine for the control of bovine leptospirosis. Efficacy has been suggested for one such multiserotype leptospira bacterin based on herd reproduction and milk production records. Fifty herds represent the base of study. Remission of herd symptomatology is established at five months after administration with the aforementioned vaccine. Additional studies to determine the efficacy of mixed bovine leptospiral bacterins are indicated.

ACKNOWLEDGEMENTS

Acknowledgement is given to Dr. R. Haight, Orange County Health Dept., Dr. G. Ott, Fromm Laboratories, Dr. W. Stouder, practitioner, for assistance and Dr. A. D. Alexander, WRAIR and Dr. D. Ivler, USC School of Medicine for assistance and guidance in the conduct of this study.

REFERENCES

LEPTOSPIROSIS IN CALIFORNIA DAIRY CATTLE


LEPTOSPIRAL SEROTYPES IN ILLINOIS CATTLE AND SWINE

L. E. Hanson,* D.V.M., Ph.D.; P. R. Schnurrenberger,** D.V.M., M.P.H.;
R. B. Marshall,*** B.V.Sc.; G. W. Scherrick,**** D.V.M.

Urbana and Springfield,* Illinois

INTRODUCTION

Since the isolation of *Leptospira pomona* from cattle¹ and swine,⁶ extensive serologic testing has been conducted with cattle and swine sera. It soon became apparent that leptospirosis was a major disease of domestic animals; however, some cattle and swine having signs associated with leptospirosis did not have *L. pomona* antibodies. In an effort to determine if other leptospiral serotypes might be present, serologic surveys of bovine and swine blood were conducted using a variety of antigens.⁵,⁷,⁹ *L. hardjo* and *L. grippotyphosa* antibodies were commonly detected in cattle sera while *L. canicola*, *L. icterohaemorrhagiae* and *L. grippotyphosa* were often found in swine sera.

Demonstration of antibodies for various leptospires in cattle and swine sera has stimulated attempts to isolate the suspected serotypes. As a result *L. canicola*, *L. hardjo* and *L. grippotyphosa* were recovered from cattle.⁸,¹³,¹⁵ *L. canicola* was the only additional serotype isolated from swine.

The objectives of this paper are to (1) indicate the current activity of five serotypes of leptospires in Illinois cattle and swine, (2) compare the reactor rates of *L. pomona* obtained each year in Illinois during a twelve-year period and (3) report isolation of *L. grippotyphosa* and *L. hardjo* from Illinois cattle in 1964.

MATERIALS AND METHODS

Swine and cattle sera were collected by practicing veterinarians and submitted for serologic testing and leptospiral antibodies to the State Diagnostic Laboratory at Urbana, Illinois. The sera were first tested with a commercial *L. pomona* plate antigen. Sera reacting to the plate agglutination test were further tested with the agglutination-lysis test for verification. Sera from herds with a history suggestive of previous leptospiral

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The authors would like to thank the following individuals who assisted in this study: Rachel Marlowe, W. R. Manuel, A. A. Shireman and M. K. Coad of the College of Veterinary Medicine. Also the authors wish to thank the personnel of the Illinois Department of Agriculture Diagnostic Laboratory.

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LEPTOSPIRAL SEROTYPES

infection when requested by the veterinarian, were also tested by the agglutination-lysis procedure using *L. grippotyphosa*, *L. canicola*, *L. hardjo*, *L. icterohaemorrhagiae* and *L. hyos* antigens. The agglutination-lysis test was conducted according to the procedure described by the Committee on Leptospirosis of the 64th Annual United States Livestock Sanitary Association. All sera causing agglutination-lysis of 50 percent or more organisms in dilutions of 1:100 or greater were regarded as positive.

In selected herds, attempts to isolate leptospires from cattle and swine urine or kidney tissues were made when high leptospiral titers were detected in sera of animals which had aborted. Voided urine samples were collected in sterile containers. Approximately a ml. of urine was inoculated intraperitoneally in gerbils or hamsters and a half ml. was inoculated into a liquid medium. Kidney tissue suspensions were made by forcing approximately one cubic cm. of kidney cortex through a sterile 2 1/2 ml. disposable syringe into liquid medium. Two dilutions were made by transferring serially one ml. of urine or kidney suspension into additional liquid media. The medium, an albumin polysorbate 80 medium containing 200 mg of 5’-fluorouracil* to inhibit growth of other bacteria, was used for cultivation of leptospires. Darkfield examinations of cultures were made weekly for approximately two months. Contaminating bacteria when present were separated from leptospiral isolates either by filtration of the culture through a .22 mm. Millipore** filter or by animal inoculations. Some contaminated cultures were inoculated intraperitoneally into gerbils and the leptospiral organisms were reisolated from the blood four or five days after inoculation. A preliminary identification of the culture was made with known antiserum using the agglutination-lysis test. Confirmation of the identity of the organisms was made by Dr. A. M. Alexander at the Walter Reed Army Institute of Research, WHO/FAO Leptospirosis Reference Laboratory.

RESULTS

Sera from 15,066 cattle and 16,903 swine were tested for *L. pomona* antibodies in 1964. The reactor rates were 5.26 percent for cattle and 1.07 percent for swine. This was the lowest reactor rate for either species during the 12 years that tests have been conducted (Table I). The highest reactor rates for both species occurred in 1955.

Sera from 2990 cattle and 671 swine, negative to the plate test, were further tested with five additional serotypes. The reactor rates for *L. hardjo* was 17.6 percent which was higher than during the two previous years when *L. sejroe* antigen had been used. In 1963 the rate was 12.4 percent for 914 sera and in 1962 it was 6.1 percent for 1431 sera. In 1964, the reactor rate for *L. grippotyphosa* was 6.1 percent and less than one percent for the other three serotypes. The distribution of cattle herds in Illinois with *L. grippotyphosa* and *L. hardjo* antibodies is presented in Figure 1.

*Hoffmann LeRoche Inc., Nutley, N. J.*

**Millipore Filter Corp., Bedford, Mass.**
TABLE I
Annual Reactor Rates of Illinois Cattle and Swine Sera
to *L. pomona* Antigen.

<table>
<thead>
<tr>
<th>Year</th>
<th>Bovine Sera</th>
<th>Swine Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Samples</td>
<td>Percent Positive*</td>
</tr>
<tr>
<td>1953</td>
<td>13,756</td>
<td>10.32</td>
</tr>
<tr>
<td>1954</td>
<td>9,677</td>
<td>13.99</td>
</tr>
<tr>
<td>1955</td>
<td>14,427</td>
<td>20.05</td>
</tr>
<tr>
<td>1956</td>
<td>18,700</td>
<td>16.17</td>
</tr>
<tr>
<td>1957</td>
<td>26,283</td>
<td>15.96</td>
</tr>
<tr>
<td>1958</td>
<td>26,391</td>
<td>12.17</td>
</tr>
<tr>
<td>1959</td>
<td>26,102</td>
<td>11.00</td>
</tr>
<tr>
<td>1960</td>
<td>19,352</td>
<td>9.04</td>
</tr>
<tr>
<td>1961</td>
<td>16,966</td>
<td>10.70</td>
</tr>
<tr>
<td>1962</td>
<td>20,856</td>
<td>11.73</td>
</tr>
<tr>
<td>1963</td>
<td>16,339</td>
<td>8.26</td>
</tr>
<tr>
<td>1964</td>
<td>15,066</td>
<td>5.26</td>
</tr>
</tbody>
</table>

*Percent of sera with agglutination-lysis titers of 1:100 or greater.

Tests conducted on swine sera detected a reactor rate of 1.7 percent for *L. canicola* and less than one percent for the other serotypes.

*L. grippotyphosa* organisms were isolated from several Illinois cattle herds in 1964. In a herd of 23 cattle, *L. grippotyphosa* was recovered from the urine collected from a cow five days after she aborted. *L. grippotyphosa* antibodies were found in another cow in the same herd which also had aborted a calf and in two cows which had retained placentas following birth of live calves. The sera of eight other animals of the herd had *L. grippotyphosa* antibodies. In another herd, isolations of *L. grippotyphosa* were made from the urine of two cows. Both cows had aborted calves within 10 days prior to the sampling. Seven sera from 30 cows of the latter herd (23.3 percent) contained *L. grippotyphosa* antibodies.

*L. hardjo* was isolated from the urine of a cow in a large herd in which 10 abortions or still-births had occurred during the previous 18 months. The organism was isolated directly from the urine in liquid medium and indirectly, from a gerbil following inoculation with the cow's urine. Serologic tests demonstrated that 47 of 124 cattle (37.9 percent) from this herd had *L. hardjo* antibodies.

**DISCUSSION**

Serologic tests indicated a wide distribution of *L. hardjo* and *L. grippotyphosa* as well as *L. pomona* serotypes throughout Illinois. The reactor rate for *L. pomona* declined from 11.73 percent in 1962 to 8.26 percent in 1963 and to 5.26 percent in 1964, while reactor rates for *L. grippotyphosa* and *L. hardjo* increased during the same period. It is possible that some of the reduction of the prevalence of *L. pomona* infection may be attributable to the widespread use of bacterins. However,
H. Herds serologically positive to *L. hardjo*. (61)

G. Herds serologically positive to *L. grippotyphosa*. (29)
*Isolations of *L. grippotyphosa*
**Isolations of *L. hardjo***

Figure 1. Distribution of *L. hardjo* and *L. grippotyphosa* positive cattle herds in Illinois.

Bacterins also were extensively used during some years when the reactor rates were high. The apparent higher reactor rates of *L. hardjo* and *L. grippotyphosa* antibodies in cattle may be a result of selection of sera from cattle showing signs suggestive of leptospirosis.

Reactor rates of *L. pomona* in swine have decreased from a high of
21.2 percent in 1955 to a low of 1.07 percent in 1964. The decline was more consistent and much greater than that observed in cattle. Reactor rates for the other serotypes also were low in swine in 1964. In Illinois leptospirosis, as reflected by serologic tests, currently appears to be less prevalent in swine than in cattle.

The pathogenicity of *L. grippotyphosa* for cattle was indicated by isolations of the leptospires from three cows in two herds following abortions. Other cattle in the same herds developed antibodies following their abortions. Previous isolations of *L. grippotyphosa* from an Illinois herd were also made from cattle following abortions.\(^8\),\(^11\)

The relationship between the presence of *L. hardjo* organisms in cattle urine and signs of leptospirosis has not been consistent. Sulzer *et al.*,\(^14\) Robertson *et al.*,\(^12\) and Clark *et al.*,\(^3\) associated infections of *L. hardjo* with signs of leptospirosis, while Roth *et al.*,\(^13\) was unable to relate presence of *L. hardjo* in a calf to signs of leptospirosis. Of the two isolations of *L. hardjo* made from Illinois cattle in 1964, one was from a member of a herd which had had 10 abortions or still-births during an 18 month period. The other was isolated from a feed lot steer with no associated signs.\(^11\)

**SUMMARY**

Serologic tests indicated that *L. pomona*, *L. hardjo* and *L. grippotyphosa* antibodies are widely distributed in Illinois cattle. Sera from 15,066 cattle examined for *L. pomona* antibodies had a reactor rate of 5.26 percent. This is the lowest reactor rate recorded in a 12-year testing period (1953-1964). Sera selected for additional testing had reactor rates of 17 percent for *L. hardjo* and seven percent for *L. grippotyphosa*. The higher rates for the later serotypes probably resulted from selection of many samples from herds in which abortions or breeding problems had occurred.

The reactor rates of the swine sera were 1.07 percent for *L. pomona*, 1.7 percent for *L. canicola*, but less than one percent for the four other serotypes.

Isolations of *L. grippotyphosa* were made from three geographically separated cattle herds. All isolations came from herds in which abortions had occurred. Three were made from cows less than ten days following abortions. *L. hardjo* was isolated from two cattle. One isolation was made from a cow from a herd with previous abortions while the other was made from a feed lot steer.

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SOME ASPECTS OF LEPTOSPIROSIS CONTROL

O. H. V. Stalheim, D.V.M., Ph.D.*

Ames, Iowa

During the past 15 or 20 years, our concept of leptospirosis has evolved from that of a rare but highly fatal disease of man and dogs caused by *Leptospira icterohaemorrhagiae* to that of a widespread but usually inapparent infection of most wild and domestic animals caused by a number of serologically distinct leptospires. The first leptospire to be isolated from cattle and swine in this country, *L. pomona*, is now well established in wild and domestic animals and is the cause of severe losses to American cattle producers. In attempts to control leptospirosis, cattle producers use 24 million doses of *L. pomona* bacterin annually. This is more than any other immunizing agent except blackleg bacterin.

Other leptospires, such as *L. icterohaemorrhagiae*, *L. ballum*, *L. canicola* and *L. hardjo*, have been isolated from cattle with and without recognized clinical signs of disease. These infections have had only localized economic significance, so far. However, they are a public health hazard, not only to those persons who come in contact with the infected animals, but also to those who seek recreation in streams and ponds contaminated by urine from infected animals.

Another leptospire, *L. grippotyphosa*, has been repeatedly incriminated by serological means as the cause of bovine abortions in Iowa and elsewhere; this organism was not isolated until recently and only after improvements were made in laboratory media for the cultivation of leptospires with fastidious growth characteristics. *L. grippotyphosa* was the cause of serious losses among cattle in the United Soviet Socialist Republic and Israel. Although it is premature yet to make conclusions, the virulence of the American strains of *L. grippotyphosa* appears to be low and comparable to that of the Danish strains.

*Leptospira hyos* and *L. autumnalis* have not yet been isolated from meat animals in this country, although high incidences of infection were found in surveys of apparently normal wild animals. Much of the bovine leptospirosis in Japan is caused by *L. autumnalis*. Swine are a suitable host for *L. hyos*. When the kidneys of 178 normal market hogs in Argentina were examined for leptospires, 45 were culturally positive. Of these 45 cultures, 35 were *L. hyos*. Therefore, we can confidently expect the isolation of *L. hyos* and *L. autumnalis* from meat animals in this country.

In addition to these infections with recognized leptospiral serotypes,

*Project Leader, Leptospirosis Research, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.*
leptospires that do not react in the standard agglutination tests have been isolated from animals.25,26 And, other animals harbor leptospires, which despite intensive efforts have not been identified.26,27 Finally, accurate estimates of the incidence and economic cost of leptospirosis in farm animals are not available despite the recommendations of this Association.28 Therefore, significance of leptospirosis in animals cannot be appraised conclusively.

Artificial immunization is an important aspect of the control of leptospirosis. Most reports indicate that vaccination of cattle or swine with killed cultures of *L. pomona* provides some protection against the clinical signs of leptospirosis.29,30,31,32 The degree of protection against development of the carrier-condition (renal leptospirosis) has not been established.32 A recent report of our Leptospirosis Committee said: "Bacterins prepared from *L. pomona* have been widely used but few critical studies on their efficacy have been reported."33

Studies on artificial immunization for the control of leptospirosis are in progress at the National Animal Disease Laboratory. The objective is the development of a safe and efficient immunizing agent which will protect cattle and swine for at least a year against not only the overt signs of leptospirosis, but more importantly, against the development of the carrier-condition. The carrier-condition may be established in inadequately immunized cattle,30,31,32 swine,34 hamsters and guinea pigs35 without any attending clinical signs including fever. In these studies, therefore, protection against renal leptospirosis was chosen as the ultimate criterion of efficacy of an immunizing agent.

The studies on immunization were necessarily preceded by a search for suitable challenge strains. Several strains of *L. pomona* were administered to different species of animals. At least two weeks later, the kidneys of the survivors were examined and cultured for leptospires.35 Different types of leptospiral disease were initiated in hamsters, mice, and pigs (Table I). The MLS and Ohio strains caused fatal infections in hamsters; they had MLD100 values of less than 50 and 150 organisms, respectively. As few as 1000 organisms of the Wickard strain regularly initiated renal leptospirosis in hamsters. The MLS strain initiated renal infections in mice, but not swine; the Ohio strain invariably infected the kidneys of swine but not the kidneys of mice. The infectious dose 100 values were determined. In subsequent experiments, the immunity of vaccinated swine or hamsters was challenged by the administration of approximately one million infectious doses of the Ohio or Wickard strains, respectively.

Experimental bacterins were prepared and administered to hamsters

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hamster</th>
<th>Mouse</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLS</td>
<td>Fatal</td>
<td>Renal</td>
<td>None</td>
</tr>
<tr>
<td>Ohio</td>
<td>Fatal</td>
<td>None</td>
<td>Renal</td>
</tr>
<tr>
<td>Wickard</td>
<td>Renal</td>
<td>Renal</td>
<td>Not done</td>
</tr>
</tbody>
</table>
and swine. The bacterins consisted of killed, whole cultures of *L. pomona* strain DM2. The cultures were grown on a shaker to nephelometric readings of 16 to 24 in a synthetic medium modified by increasing the content of polysorbate 80 to 0.05 percent. After the bacterial density was determined by direct microscopic counts, the cultures were diluted with 0.02 molar phosphate buffer (pH 7.3) to a standardized concentration of $10^8$ organisms/ml and inactivated by the addition of quaternary ammonium (0.001 percent). At least two weeks after administration of the bacterin, the immunity of the vaccinated hamsters and swine was challenged as described above.

The experimental bacterin protected hamsters against the lethal effects of the MLS strain of *L. pomona*, but not against infection and initiation of renal leptospirosis (Table II).

**TABLE II**

Protection by Bacterins Against Fatal but Not Renal Leptospirosis*

<table>
<thead>
<tr>
<th>Dilution of bacterin</th>
<th>Challenge strain:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MLS</td>
<td>Wickard</td>
</tr>
<tr>
<td>1:40</td>
<td><strong>0/5</strong></td>
<td>*<strong>8/10</strong></td>
</tr>
<tr>
<td>1:80</td>
<td>0/5</td>
<td>5/5</td>
</tr>
<tr>
<td>1:160</td>
<td>1/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Nonvaccinated controls</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

*Two weeks after one ml. of diluted *L. pomona* bacterin was administered to hamsters, their immunity was challenged by approximately $10^6$ or $10^8$ *L. pomona* of fatal (MLS) or renal (Wickard) strains. Numbers of hamsters which died** or had renal infections***/the number challenged.

The experimental bacterin and a commercially produced *L. pomona* bacterin were administered to swine by intramuscular injections in doses of five milliliters. As shown in Table III, neither of the bacterins protected swine against renal infections with *L. pomona* strain Ohio. Vaccinated and nonvaccinated swine shed similar numbers of leptospires/ml. of urine.

**TABLE III**

Failure of Bacterins to Protect Swine Against Renal Leptospirosis

<table>
<thead>
<tr>
<th>Immunizing agent</th>
<th>No. carriers/No. challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental bacterin, 5 ml.</td>
<td>8/8</td>
</tr>
<tr>
<td>Commercial bacterin, 5 ml.</td>
<td>16/16</td>
</tr>
<tr>
<td>None, controls</td>
<td>70/70</td>
</tr>
</tbody>
</table>

Although the exposures of the vaccinated animals in these studies were probably greater than that under natural exposures, the results indicate that killed *L. pomona* bacterins do not protect against leptospiral infection and initiation of the carrier-condition.
REFERENCES


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REPORT OF THE COMMITTEE ON LEPTOSPIROSIS


This year your Committee dealt with those aspects of leptospirosis most in need of continued attention, mainly diagnosis and control. Previous recommendations of this Committee were reviewed and considered and it was concluded that present viewpoints and findings do not conflict with those previously stated.

Leptospiral antibodies demonstrated in the sera of cattle indicate that a number of leptospiral serotypes are present in the United States. These serotypes include *Leptospira pomona*, *L. canicola*, *L. hardjo*, *L. icterohemorrhagiae*, *L. autumnalis* and *L. ballum*.

Isolations of *L. hardjo*, *L. grippotyphosa*, *L. icterohemorrhagiae*, *L. canicola* and several other still unidentified leptospiral organisms have been accomplished from cattle but not all have necessarily been associated with clinical disease. Similar serological findings indicate the presence of antibodies for a number of serotypes in swine with fewer serotypes isolated. The clinical significance of infections due to the various serotypes other than *L. pomona* should be established before the magnitude of the problem can be determined.

Further data must be obtained on the distribution, occurrence, and association with clinical disease before sound recommendations may be made for serotypes other than *L. pomona*.

The Committee recommends that the Agricultural Research Service and diagnostic laboratories investigate the incidence and where possible the clinical history of outbreaks associated with the various serotypes isolated in cattle and swine.

The need for a simple test to accurately detect the carrier animal is of utmost concern to the Committee. Present serological methods indicate only that the animal has had exposure at some time to leptospirae and do not necessarily indicate the animals that are spreading the disease.

Pathogenic leptospires commonly localize in the renal tubules of animals (carrier condition), where they multiply, and from where they are shed in the urine (shedder state). Thus, the infection is spread to other animals and man. The Committee therefore urges further studies on immunizing agents for the protection of animals against both infection and the carrier condition.

The Committee wishes to recognize and is highly pleased that its recommendation regarding testing and efficacy of various biological
products used for immunization has been undertaken by a control agency. Committee members are fully aware of the many problems facing the veterinarians, livestock owners and regulatory officials as a result of this carrier status disease and therefore urge that some unbiased agency fully investigate the possible role of chemotherapeutic agents for the elimination of the carrier condition.

Increased support for research is essential in the diagnostic, immunologic, epizootologic and chemotherapeutic aspects of the disease is necessary for a better understanding of leptospirosis.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this Committee continue.
COMBINED VACCINES FOR DAIRY CATTLE

Robert F. Kahrs and James A. Baker*

Ithaca, New York

In order for dairy herd health programs to be meaningful and economically rewarding, optimum management practices must be supplemented with vaccination against certain specific infectious diseases. Maximum efficiency in the utilization of vaccine resources and farm labor requires that several vaccines be administered simultaneously. The objectives of this study were as follows: (1) to determine if it is possible to combine vaccines of known viral and leptospiral conditions into a single dose, (2) to determine if it is feasible to recommend routine use of such vaccines on New York State dairy farms, and (3) to outline the methodology involved in evaluating such combinations.

MATERIAL AND METHODS

The field trial: The data reported here cover the period from December 4, 1961 to January 15, 1965. Three hundred ninety-one cattle vaccinated with combined vaccines and seven hundred ninety-six unvaccinated herdmate controls in nine New York dairy herds were studied clinically and serologically.

Vaccines and vaccination: Non-pregnant cattle were vaccinated intramuscularly with one of fourteen lots of combined vaccine, the components of which were provided by biological manufacturers or prepared at the Veterinary Virus Research Institute. The combined injection contained attenuated living vaccines for infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), bovine parainfluenza-3 (BPI-3), a Mycoplasma type organism\(^6,7\) which was later deleted, and an inactivated bacterin for *Leptospira pomona* (*L. pomona*). The individual components of the combined vaccine were occasionally altered as needs for improvement became apparent. All other procedures were consistent throughout the experiment and the comparisons between vaccinated and control cattle were based on the assumption that the vaccinated cattle comprised a relatively homogenous population.

On the first visit to a farm, one-third of the cattle between four months and five years of age were vaccinated with the combined vaccine and thereafter one-third of all calves reaching four to six months of age

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The authors are grateful to Dr. D. S. Robson, Professor of Biological Statistics, Cornell University, for assistance in statistical treatment of the data.

This work was supported by the Office of Naval Research.
were vaccinated and the remaining two-thirds were designated as control animals.

**Serum collection and serological tests:** Vaccinated and control cattle were bled at the time of vaccination and again 21-60 days later. In addition, each experimental animal was bled annually, and following abortions and other sicknesses. The serums, separated by centrifugation at 1000-3000 rpm. for 10-20 minutes were stored at -10°C until tested. All serums were heated for 30 minutes at 56°C immediately prior to testing.

The BVD serum neutralization (SN) test was performed on all serums in primary or secondary embryonic bovine kidney tissue cultures using 30-300 TCID<sub>50</sub> of the seventh passage BVD virus strain C24V, according to the method of Coggins.1 Undiluted serum (two or three tubes per dilution) was used for preliminary screening. Serums neutralizing the cytopathogenicity of the test amount of virus were considered BVD positive and those which failed to neutralize were considered negative. Positive serums, when indicated, were titrated using fourfold serum dilutions from undilute (1:2 accounting for dilution by addition of virus suspension) to 1:32,768 and 50 percent end points were estimated on the log<sub>10</sub> scale by the Spearman-Karber method.2

All serums, undiluted, were examined for IBR antibody by the IBR SN test using IBR virus strain Colorado I, seventh passage tissue culture passage in a dosage of 30-300 TCID<sub>50</sub> per tube. Each serum-virus mixture was incubated at 37°C for one hour prior to inoculation into two or three tubes of primary or stabilized secondary bovine kidney cell cultures which were then examined for cytopathology at 72 hours. The appearance of any foci of IBR degeneration in the cultures was regarded as criteria for infection. Serums which neutralized the virus were considered IBR positive. Serums which allowed viral cytopathogenicity were ruled IBR negative. Titers were determined on IBR positive serums by the same test, using twofold serum dilutions from undilute (1:2 from dilution by virus suspension) to 1:64 and log<sub>10</sub> 50 percent end points were estimated by the Spearman-Karber technique.

In order to study the response to vaccine, and to detect the presence of disease in the experimental herds, 4,161 blood samples were collected and tested. The results of serological tests provided the only means of evaluating progress in the stepwise improvement of the combined vaccine, and provided continued retrospective surveillance for evidence of vaccine spread. Initially, all serums were tested for antibody specific to the five vaccine components,4 but as serums accumulated, the task of testing all serums for all agents became prohibitive and the serums were tested only for IBR and BVD.

**Criteria for clinical comparison of vaccinated and control animals:** All farms were visited at approximately three-week intervals, the herdsman questioned regarding herd health, and barn sheets left on which were recorded any sickness including abortions, retained placenta, mastitis, diarrhea, pinkeye, and calfhood diseases. The incidence of each of the above conditions was computed for vaccinated and control animals.
and these data were examined by Chi-square analysis of fourfold (2 x 2) tables.

RESULTS

Clinical observations regarding vaccine safety: Seven slight local swellings at the site of inoculation (six vaccinates and one placebo inoculated control) and one case of acute postvaccination urticaria were observed. Seven cattle in one herd (three vaccinates and four controls) had elevated temperatures of undetermined origin on the fourth day following vaccination. Aside from the above described observations, no clinical evidence of untoward vaccine effects was apparent and it was concluded that the combination vaccine was free of any danger beyond the normal hazards associated with the use of any biological product.

Clinical evaluation of vaccine efficacy: No experimental herds experienced recognizable outbreaks of diseases attributable to the infectious agents against which the vaccine components were prepared. Nevertheless the hypothesis of vaccine effects upon inapparent or undiagnosed disease was tested by comparing vaccinate and control incidence of various manifest signs of disease. There was no significant difference between vaccinated and control animals in the incidence of mastitis, acute septic mastitis, diarrhea, winter dysentery, keratoconjunctivitis, undiagnosed illness, infertility, or the sale of cattle for all reasons.

There was a significant difference (favoring the vaccinated animals) in the incidence of abortion, retained placenta and calf septicemia in offspring of vaccinated animals. The clinical observations in vaccinated animals and unvaccinated contact herdmate controls are summarized in Table I.

TABLE I
The Comparison of the Incidence of Naturally Occurring Disease Conditions Which Appeared in Vaccinated and Unvaccinated Herdmate Control Cattle During the Three-year Observation Period

<table>
<thead>
<tr>
<th>Symptom or Condition</th>
<th>Total Cases Observed</th>
<th>Incidence (new cases/population at risk in three years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vaccinated</td>
</tr>
<tr>
<td>Mastitis</td>
<td>264</td>
<td>.3173</td>
</tr>
<tr>
<td>Acute septic mastitis</td>
<td>19</td>
<td>.0221</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>240</td>
<td>.2123</td>
</tr>
<tr>
<td>Winter dysentery</td>
<td>217</td>
<td>.1867</td>
</tr>
<tr>
<td>Keratoconjunctivitis</td>
<td>23</td>
<td>.0128</td>
</tr>
<tr>
<td>Undiagnosed illness</td>
<td>52</td>
<td>.0486</td>
</tr>
<tr>
<td>Infertility</td>
<td>103</td>
<td>.0972</td>
</tr>
<tr>
<td>Abortion</td>
<td>28</td>
<td>.0078</td>
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<tr>
<td>Retained placenta</td>
<td>60</td>
<td>.0286</td>
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<tr>
<td>Calf septicemia in</td>
<td>13</td>
<td>.0026</td>
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N.S. = not significant
Attempts to determine the etiology of abortions and neonatal mortality by the usual bacteriological, virological and serological methods resulted in mold isolations from three aborted fetuses. Virus neutralization tests for IBR and BVD, performed on sera collected before abortion, at the time of abortion, and again 30 days following abortion indicated that there was no significant rise in serum titer in 16 abortions. These findings tend to exonerate these viruses as the etiologic agents of these abortions but this interpretation is open to question. No convincing evidence either to indict or to rule out BPI-3 or other agents as causes of abortion was found.

Serological studies regarding vaccine safety: Serum neutralization tests on contact herdmate control animals associated with cattle vaccinated with the first seven vaccine combinations indicated a possibility of shed and spread of BVD vaccine from vaccinated to control animals. 17.6 percent of these unvaccinated animals developed BVD antibody within a month after vaccination of their cohorts. This assumption was strengthened by the finding that the seventh BKC passage Mycoplasma vaccine component contained noncytopathogenic BVD virus and thus the vaccinates had been receiving an extraneous BVD virus of low attenuation. The Mycoplasma vaccine was eliminated from the combination after use on 209 animals and was never replaced. The hypothesis of a relationship between the Mycoplasma vaccine and the appearance of BVD antibody in unvaccinated control animals was tested by comparing this character in controls contacting animals vaccinated with the Mycoplasma component, with controls later vaccinated with combined vaccine from which this component had been deleted. Forty of 227 unvaccinated susceptibles (17.6 percent) in contact with Mycoplasma vaccinated animals developed BVD antibody. Only six of 106 (5.7 percent) of susceptibles in contact with animals vaccinated with combined vaccine excluding the Mycoplasma vaccine component developed BVD antibody. Chi-square analysis of these data indicated there was a significant difference ($X^2 = 8.6828 \ P < .005$) between the two vaccines, and, although the possibility of natural infection cannot be ruled out, it was assumed that this extraneous virus was involved in at least some of the asymptomatic BVD infections which occurred among unvaccinated control cattle.

Serological surveillance of contact controls indicated minimal spread (less than five percent) of IBR vaccines in all combinations but one. The IBR component of this combination was 14th passage material and was of such low attenuation that 32 of 74 susceptible contact control animals (43.2 percent) developed IBR antibody.

No apparent clinical signs were evident in contact control animals which developed either BVD or IBR antibody.

Serological observations regarding vaccine efficacy: Of the 391 animals vaccinated with combined vaccine, only 174 (44.5 percent) were negative for BVD antibody at the time of vaccination. All susceptible animals which received the combination which included the Mycoplasma vaccine (and thus contained the extraneous unattenuated BVD virus) responded with 100
percent conversion of serological status to BVD positive. The BVD response to combinations administered after deletion of the extraneous BVD virus was 86.9 percent with complete lack of efficacy in one group acting to lower the overall response rate.

Three hundred thirty-two of the 391 vaccinates were negative for IBR antibody at the time of vaccination and 182 (51.62 percent) had developed detectible IBR antibody when tested 21-60 days later. This IBR response rate varied from 6.25 percent efficacy in early attempts to 100 percent in cattle on which less attenuated IBR vaccine was used.

IBR vaccine responses as measured by serum neutralization tests were subject to variation, depending upon the time interval between vaccination and the collection of the postvaccination serum sample. IBR SN tests on serums collected annually from all herds indicated that some vaccinated animals negative 21-60 days following vaccination appeared positive on later tests, while this seroconversion was less apparent among control animals. Sixty of 115 vaccinated animals (52.1 percent) negative on the first postvaccination test (and thus regarded as vaccine failures) were found positive when routinely tested four to 30 months later, while only 18 of 406 originally negative control animals (4.5 percent) developed IBR antibody at a later date. This difference was highly significant \( P < .005 \) when examined by Chi-square test of fourfold tables (Chi-square = 140.05). Limited further study of nine IBR vaccinated cattle indicated that this delay was real, since sequential serum samplings of nine vaccinated calves indicated a response rate of 22 percent at three weeks, 44 percent at six weeks and 56 percent at 36 weeks.

The persistence of IBR antibody following successful vaccination indicated that approximately 90 percent of animals were still immune up to 36 months following vaccination. Almost 100 percent of animals retained BVD antibody but the validity of this information and an indicator of vaccine effect is negated by the discovery of extraneous BVD virus in the mixture used in the first half of the field trial.

**Combined vaccine administered at the time of Brucella Abortus vaccination**: Seventy-one calves received *B. abortus* strain 19 vaccine at the same time that they were inoculated at another site with combined vaccine. To determine if the combined vaccine inhibited the response to the *B. abortus* vaccine, serums were taken from 50 of these calves and 88 contact controls which received only *B. abortus* vaccine. When tested for *B. abortus* antibody by means of a tube agglutination test, serums from all 138 were free of detectible *B. abortus* antibody (titer < 1:25) before vaccination. Thirty days following vaccination, all calves in both groups had agglutination titers of 1:50 and there was no significant difference between the vaccinated and control animals. Thus it was concluded that *B. abortus* vaccine could be used simultaneously with the combined vaccine satisfactorily.
SUMMARY AND CONCLUSIONS

The combined vaccines studied were safe when used on healthy dairy cattle which were not under conditions of severe stress or incubating infectious agents. Serological tests provided a continual means of evaluating vaccine safety and efficacy, and when the variables related to serological vaccine evaluations were manipulated advantageously, both safety and efficacy were demonstrated.

Several encouraging aspects of combined vaccines were apparent from this work.

1. The duration of immunity as evidenced by antibody persistence as long as three years following IBR vaccination was longer than previously suspected and suggests that calfhood vaccination is a reasonable approach to control of this disease.
2. The positive effect of vaccination prior to pregnancy in reduction of abortion suggests that this approach to the abortion problem has considerable merit.
3. The suggestion that neonatal morbidity and mortality can be reduced by maternal protection induced by early vaccination of animals raised for breeding purposes offers tremendous potential for further research.

The authors held the opinion that there is a bright future for combined vaccines for dairy cattle, although further work will be required to define and incorporate those components for the best combination.

REFERENCES

THE DIAGNOSIS OF TRICHOMONIASIS IN THE BULL

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Bovine trichomoniasis has been diagnosed in most states in the United States at one time or another, in most countries in Europe, in Australia, and several countries in Asia. It was recognized as a reproductive problem in Europe as early as the 1920's, and in this country as early as 1932. At this time and in the 20 years following it received much attention in the dairy industry where it was diagnosed most frequently. With the coming of artificial insemination and subsequent reduction in the holding and use of bulls in individual herds, trichomoniasis has been diagnosed with much less frequency in the dairy industry in the last 10 years. As a result of this apparent decline, a gradual decline of interest has also occurred. This is indeed a serious situation, for trichomoniasis is perhaps as prevalent in this country today as at any time in our history. A recent report revealed that the disease is widespread in the beef herds of the Rocky Mountain West. Seven and one-half percent of 828 beef bulls tested in herds ranging from Montana to Arizona were found to have trichomoniasis. If comparable diagnostic efforts were made in other parts of the West, it is likely that trichomoniasis would be recognized as more of a problem in these areas also. Trichomoniasis is not confined to beef animals, however. It is still diagnosed in dairy animals, and with its tremendous potential for spreading can become a problem with disastrous results at any time.

The need, therefore, for knowledge and know-how in the diagnosis of trichomoniasis is as great today as ever. The emphasis may be shifting somewhat from dairy to beef animals, but while the disease exists in the proportions that it does, there should be great concern by all who are associated with large animal practice and disease control.

It is not the purpose of this paper, however, to establish the importance of trichomoniasis, but rather to re-emphasize it and to call for renewed interest and increased efforts in the application of earnest diagnostic procedures. Those who have had experience with the disease do not need to be continually reminded. The literature is full of case histories reporting serious reduction in calf crops, the loss of breeding animals through sterility as a result of pyometra, and unsuccessful attempts to live with the disease. And yet we continue to ignore its destructive potentiality. Why? Perhaps the insidious nature of the disease is partly responsible. We tend to respond to the more spectacular. There also seems to be a reluctance by many to attempt diagnosis, for they feel that present diagnostic methods are too time consuming, unreliable, and repeated samples must be taken to insure accuracy. Thus, the effort and expense

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of diagnosis leads to procrastination until action is forced by the seriousness of the situation.

It is, therefore, the purpose of this presentation to point out that there are methods available by which the diagnosis of trichomoniasis may be made with very little effort, a high degree of accuracy, and with a minimum of time involved.

Diagnosis in the bull is accomplished by demonstration of the live organism which is found in the sheath and on the glans penis in relatively small numbers which fluctuate considerably. This environment may sustain the trichomonad for the life of the bull but does not support the luxuriant growth that occurs in the uterus. The bull might in a sense be classed as intermediate or reservoir host in that the organism apparently does him no damage, and he is the instrument by which the organism is transferred from definitive host (the cow) to definitive host. This relatively poor existence of the trichomonad in the bull cannot be expected to cause a systemic reaction of sufficient degree to be exploited for diagnosis, and indeed all tests based on systemic reactants have been highly unreliable, if not total failures. With the present knowledge of the disease it is only wishful thinking that a blood test might be developed. We must therefore, continue to rely on the demonstration of the organism either by direct methods or by culture.

There are two basic steps in the diagnosis of trichomoniasis: (1) recovery of the organism from the bull, and (2) visual demonstration of the organism.

Recovery of the trichomonad from the sheath and glans penis has been accomplished by three well publicized methods—cotton or gauze swab, aspiration of smegma by pipette from the glans, and douche of the preputial cavity with saline. Regardless of the method used, most attention should be given the area of the glans penis, for here the organism occurs in greatest number. Proponents of the swab and pipette methods feel that there is one advantage of this method. They may be more selective in the area which they sample and avoid dilution and contamination by extraneous debris. The douche method does not allow this selection, and this debris must be reckoned with. Trichomonads, however, do occur in significant numbers in other areas of the sheath. The study of Hammond and Bartlett revealing the greatest concentration of trichomonads in the glans penis area also revealed significant concentrations in four other areas. Of the three bulls studied, 77, 42, and 55 percent of the total trichomonads recovered were found on the glans. Therefore, any method which concentrates on this area alone leaves 23, 58, and 45 percent of the trichomonads in the sheath. A thorough douche of the entire area recovers many of these trichomonads. In another study on a like number of bulls by Fitzgerald et al., approximately nine times as many organisms were recovered by douche as by pipette. There seems to be little doubt that more organisms may be recovered by the douche method than by the pipette and swab methods.

Visual demonstration of the organism in smegma and debris collected by the swab and pipette methods, which usually amount to less than one cc
in volume, are made by observing this material directly under low power of the microscope. The efficacy of these methods is proportional to the thoroughness and skill of the collector and to the thoroughness with which the sample is searched under the microscope. When trichomonad populations are low, accuracy is low; and several repeats are necessary to increase the chances of finding the organism. These methods have the advantage of requiring little equipment and of obtaining a rather quick diagnosis since no waiting period is necessary for culture. This latter advantage is lost, however, when several samples must be taken over a period of several days or weeks.

The douche method requires concentration of the trichomonads by centrifugation, inoculation of the sediment into culture medium, and examination three to four days later by microscope for diagnosis. Properly executed and using proved medium, one sample is sufficient for diagnosis in most cases. The need for repeat sampling is easily determined, and these indications will be discussed later.

**Evolution of the Douche-Culture Method**

The present douche-culture method as used in our laboratory is not a method hastily conceived and proved by a few trials. It has evolved over a period of several years. It was reasoned early that the best method of finding the trichomonad was to look where it was in greatest concentration; and thus the douche method of recovery of the organism was chosen. Concentration of the organisms from the sample by centrifugation, however, also concentrates the debris which is present in extremely varying amounts. When a little debris is present, the trichomonad may be easily spotted by a direct examination under low power of the microscope. When much debris is present, the organism is difficult to find if the concentration is low. Thus, one is confronted with the same problem as occurs with the swab and pipette method, and the only alternative is repeated samples in hope that you may catch the fluctuating population at a higher level or the debris at a low level. It was, therefore, decided in our laboratory to supplement the douche direct examination with cultures of the remaining douche sediment. Various media and inoculation techniques were tried in a determined effort by Dr. P. R. Fitzgerald. A modification of the medium of Plastridge in which Dr. W. Plastridge reported that he could start a culture of trichomonads from one organism was finally chosen as the most ideal. The medium is liquid and simple to prepare, and it contains enough agar to cause the inoculum to float on its surface. The addition of penicillin and streptomycin inhibits bacterial growth, or confines it to a small area on the surface of the medium. Because of its size and activity, the trichomonad in its search for the proper oxygen tension migrates to the bottom of the culture tube where growth starts in medium that is unaffected by and free of the inoculated debris. This douche-direct-culture technique in which the douche fluid was instilled into the sheath by glass pipette and rubber bulb and drained into a wide mouth jar was used effectively for several years. On occasions, however, the antibiotics were overwhelmed by bacteria and the cultures overgrown,
largely as a result of bacteria washed from the lower preputial cavity and orifice. To eliminate this problem, a rubber rectal tube equipped with an enema hose clamp was substituted for the glass pipette and allowed to remain in place during the douche massage. After massage the fluid was then drained through the tube eliminating contact with the highly contaminated sheath orifice. Bacterial overgrowth now occurs in less than two percent of the samples taken.

Application of the Douche Method

The douche-culture method has been used under almost every field situation which may be encountered and has been used with excellent results on many experimental animals. Its use in testing 1,200 beef bulls for trichomoniasis has been reported. Early experimental use of the technique in the field revealed that the culture was much more accurate than the direct examination, which was 60 to 75 percent accurate. As a result direct examinations in the field were discontinued in most instances. It was, however, more expedient to use direct examination on samples taken from bulls undergoing experimental treatment. Culture was used to supplement direct examinations which were negative.

Tests on Known Infected Bulls

A tabulation of 227 samples taken from 83 known infected bulls revealed 98 percent of 170 of these samples were positive by culture. Of 163 of these samples examined by the direct method, 73 percent were positive. A complete breakdown of the samples is as follows: 62 samples were positive by both direct and culture, 41 were negative by direct and positive by culture, one positive by direct but negative by culture, two negative direct and negative culture, 64 no direct examination and positive by culture, and 57 positive by direct and no culture.

Four bulls were sampled ten or more times, five bulls five or more times and 23 bulls three or more times, resulting in multiple sampling on 44 bulls. Multiple samples on individual bulls ranged from several days apart to several months apart, which allowed sampling at various stages of fluctuation in the trichomonad population. As a test for culture methods, it is felt that a relatively few samples from a large number of infected bulls is of greater value than a large number of samples from a few bulls. Also many samples were taken from one to two weeks after experimental treatment for the disease at which time the trichomonad population was very low. The two samples negative by direct and by culture were such samples. Subsequent samples taken a week later from these two bulls were positive by culture.

Douche-Culture Technique

Sampling Equipment Unit

1. One five-ounce rubber bulb (rectal syringe) with two-inch glass tubing adapter.
2. One rectal tube with two-holed tip size 26f, 18–20 inches long with clamp, such as clamp from hot water bottle hose.
3. One serum bottle containing 100-125 cc physiological saline.

The bulb and tube can be packaged very handily in a No. 3 paper sack and autoclaved. By autoclaving the saline, also, and stoppering it tightly, the sampling units may be stored for several weeks or months and are, therefore, ready at a moment's notice.

Sampling Procedure

1. With the use of the tube, fill the bulb with the saline.
2. Insert the rubber tube (detached from bulb) its full length into the sheath.
3. Maintain closure of the sheath tightly around the tube with thumb and index finger of one hand dorsal to the tube, and introduce the saline through the tube from the bulb with the other hand. Partial closure of the clamp can be effected with the little finger of the hand clamping the sheath while the bulb is being discarded.
4. Massage thoroughly the glans penis through the sheath, and to a lesser extent the other regions of the prepuce using 50 to 75 vigorous strokes and breaking the rhythm frequently to prevent ejaculation.
5. Open the clamp and allow the fluid to drain back into the bottle slowly withdrawing the tube as the fluid drains.
6. If direct examination is to be made, the sample should be taken to the laboratory and examined as soon as possible while the trichomonads are at their highest state of activity. If culture alone is employed, the sample may be refrigerated and held several hours before examination.

Examination of the Sample

1. Concentrate the trichomonads by centrifuging the sample at 2,000 rpm for 10 minutes in 50 to 100 cc centrifuge tubes. Decant the supernatant leaving a total of about 15 cc of the concentrate in the bottom of the tube (or tubes).
2. Transfer the concentrate to a 15 cc centrifuge tube and recentrifuge at 2,000 rpm for 10 minutes.
3. Carefully decant the supernatant leaving a few drops on the sediment so that, upon shaking, the sediment is just fluid enough to pour. There will usually be one to two cc of sediment in the bottom of the tube.
4. Make a direct examination of this material by placing a drop of the sediment on a slide; cover with cover glass and examine under low power on the microscope.
5. If no trichomonads can be found by direct examination, the remainder of the sediment should be cultured by carefully pouring approximately one cc of the sediment on to the top of 8-10 cc of modified Plastridge medium in a screw cap culture tube to which 1,000 units of penicillin and one milligram streptomycin (both in saline) per cc of medium have been previously added.
6. Incubate the tube for four days at 37° C and examine under low power of the microscope the bottom drop of medium, which has been removed with a nine-inch disposable capillary pipette. The drop is best examined without coverslip, and trichomonads if present are readily apparent. Often growth starting in the bottom of the tube may be apparent grossly as a slight opacity in the clear medium.

**Precautions in Use of Douche-Culture**

These precautions must be followed religiously in using the douche-culture method:

1. The douche sample after collection should never be allowed to become warmer than body temperature. Trichomonads die rapidly in warm saline.

2. Samples after collection should be refrigerated or cooled if held more than one hour before examination. By cooling immediately after collection, samples may be held up to 24 hours before processing; although it is advisable to process them as soon as possible. This procedure allows ample time for transportation to the diagnostic laboratory.

3. Modified Plastridge medium should contain serum not over two weeks old. Medium without serum may be stored refrigerated for long periods without change, and serum may be added just prior to use. Commercial serum is not satisfactory.

4. At the end of the culture period all samples that have bacterial or yeast growth throughout the tube should be repeated. Those in which the inoculum has sunk to the bottom of the tube should be repeated. Those in which the bull has ejaculated or urinated should also be repeated. Ejaculation can be avoided by taking care in massage. Urination is occasionally a problem in early morning sampling and may be remedied by withholding water the night before. If there is a question on any culture, repeat it!

Cultural procedures are not practical for the general practitioner who may make an occasional test, and it is recommended that he collect the sample and have it processed in a diagnostic laboratory. The culture technique, however, should be very valuable to organizations such as artificial insemination centers, which usually have a small laboratory available. Also in organizations of this type where large numbers of bulls may require frequent testing, modifications of the technique which may eliminate some of the equipment are possible such as the use of the small pump to instill the saline instead of the rubber bulbs.

The douche-culture method of testing for bovine trichomoniasis is a technique with which all veterinary diagnostic laboratories should be familiar and is a service which they should offer. It has a high degree of accuracy when the previously mentioned precautions are taken. It is simple enough that large institutions and commercial organizations can adapt it to the facilities which they have. It provides the general
practitioner with a simple means of obtaining material to be tested and places the main responsibility of diagnosis on the diagnostic laboratory. It must be remembered, however, that diagnosis in the laboratory can only be as good as the sample obtained.

REFERENCES

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) has been classically described as a mild to severe upper respiratory infection. Since these initial reports, others have described lesions of vulvovaginitis, balanoposthitis, keratoconjunctivitis, encephalitis and a fatal disease of newborn calves. Our interest was turned to this virus as a cause of enteritis in the bovine when a strain of IBR was isolated from a Peyer's patch of a calf which had died with typical lesions of mucosal disease. A second virus which was related to the bovine viral diarrhea-mucosal disease group (BVD-MD) was isolated from the bone marrow of this animal by Dr. W. A. Malmquist of the National Animal Disease Laboratory.

These findings led us to postulate that the newly isolated strain of IBR and possibly many other strains, either alone or in combination with viral diarrhea agents, were able to replicate and produce lesions in the alimentary canal of the bovine. The findings also suggested that the isolate was capable of a degree of generalization not generally associated with other strains of IBR.

The literature is not without reference to diarrhea as a part of the IBR syndrome. As early as 1955 Miller reported cases of IBR associated with diarrhea. Chow et al., and Jensen et al., similarly reported diarrhea as an inconstant symptom of the disease. McKercher et al., have reported that diarrhea is rarely if ever, present in cases of IBR. In a later publication, however, McKercher and Straub describe the isolation of IBR from range cattle where diarrhea seemed to be the predominant symptom. The authors suggested that the deaths in this outbreak may be associated with concurrent infections of mucosal disease or malignant catarrhal fever. A viral diarrhea agent was not isolated from this outbreak nor were serological studies conducted to determine the presence of BVD-MD neutralizing antibodies. Baker et al., in a study of experimental IBR infection in calves, were able to induce fatal cases of the disease with systemic infection. Virus was recovered from the liver, spleen,
INFECTIOUS BOVINE RHINOTRACHEITIS

and kidneys and lesions in the mouth, esophagus and forestomach were present. The authors suggested that the name "IBR-IPV" is an unfortunate designation for this virus in view of their findings.

This entire study was directed towards elucidating the pathogenesis of the disease produced by the new isolate of IBR. Towards this end the virus was cloned and compared to other strains by reciprocal cross neutralization tests. After these characteristics were established, a fluorescent antibody tracing method was devised which would allow us to follow the distribution of viral antigen in tissues of infected animals.

MATERIALS AND METHODS

Cell cultures. Bovine testicle cell cultures were used throughout this study and were suitable for primary isolation and reisolation trials, plaque assays, and fluorescent antibody studies of infected cells. Details of culture preparation, mediums and solutions used have been previously reported.6

Plaque assay. In this study the plaque assay was utilized as a method of purification and as an indicator system in serum neutralization tests. Calf testicle cells were propagated in 60 mm Falcon plastic petri dishes* until complete monolayers were formed. Generally, an initial seeding of $1 \times 10^6$ cells per ml of medium would result in good monolayers in three to four days when five ml of medium-cell suspensions per dish were added. After monolayers had formed they were washed with saline G19 and one ml aliquots of virus dilutions were inoculated into three replicate plates and allowed to adsorb for one hour. After this adsorption period, excess inoculum was decanted and the plates were overlaid with Eagle's medium with one percent Noble agar.** The cultures were allowed to incubate in an inverted position for four days at 37 C in a five percent carbon dioxide atmosphere. A second agar overlay containing $1/10,000$ neutral red was added after four days in order to more clearly differentiate plaques.

Virus strains. In addition to the newly isolated IBR strain described in this study, the Colorado (Cooper) and Los Angeles (California-5) strains*** of IBR were used in reciprocal cross-neutralization studies. On receipt of these later two strains both were passed twice in bovine testicle monolayers. All strains were passed through two plaque selection passages. After the second plaque selection, the three strains were propagated on testicle cell monolayers nurtured with Eagle's medium devoid of serum in order to secure a working lot of virus.

Rabbit hyperimmunization. Each strain of cloned virus was used to hyperimmunize two young rabbits. Live virus containing approximately $7 \times 10^6$ PFU (plaque forming units) per ml was given intravenously (two

*Falcon Plastics, Los Angeles, California.
**Difco Laboratories, Inc., Detroit, Michigan.
***Obtained from Dr. C. E. Phillips, National Animal Disease Laboratory, Ames, Iowa.
ml) at four day intervals. Hyperimmunization was carried on for six weeks. The rabbits were bled seven days after the last injection. A one ml aliquot of virus dilution containing 1000 PFU's was added to one ml aliquots of five-fold serum dilutions. The serum-virus mixtures were allowed to incubate at 37 C for 30 minutes at which time 0.2 ml aliquots of each serum-virus mixture was inoculated on three monolayers of bovine testicle cells grown in 60 mm diameter plastic petri dishes. Adsorption was carried on for one hour after which time five ml of overlay medium was added. Controls consisted of testing mixtures of virus ($10^3$ PFU/ml) against equal volumes of non immune rabbit serum, and saline. After four days of incubation at 37 C, plaque counts were made and the virus neutralization index recorded as the reciprocal of the final serum dilution neutralizing 100 percent of the plaques.

**Calf hyperimmunization.** A six month old male calf was castrated in order to procure testicle cell monolayers for virus propagation and subsequent hyperimmunization of the same calf. The IBR-ISU-1 strain reached titers of approximately $10^7$ PFU/ml. The calf was initially inoculated intravenously with $10^7$ PFU by the intravenous route and allowed a 21 day period to progress through the experimental disease. On days 21, 28, and 35 postinoculation, the calf was given 10 ml of virus ($10^7$ PFU/ml) intravenously. On days 28, 35, and 43 the calf was injected with a mixture of virus (five ml) and complete Freund's adjuvant (five ml) at two sites adjacent to the prefemoral and prescapular lymph nodes. The calf was bled for serum harvest seven days after the last injection.

**Fluorescent antibody methods.** Recovery of globulins from the hyperimmunized calf was accomplished by ammonium sulphate precipitation at 4 C. After two precipitation cycles, the precipitated globulins were redissolved in 0.15 M NaCl to a protein concentration of one percent.

Conjugation of fluorescein isothiocyanate to the globulin was done according to the method described by Marshall *et al.* Five ml of a 0.5 M carbonate-bicarbonate buffer at pH 9 was added to 45 ml of the globulin solution. Fluorescein isothiocyanate was added to the mixture at the rate of 0.05 mg per mg of protein. Conjugation was allowed to proceed overnight with constant agitation.

Unconjugated dye was removed from the fluorescein-globulin mixture by the method of Killander *et al.* This consisted of passing the conjugated globulin solution through a column of Sephadex G-25* packed to a volume of 2 x 5 cm per ml of conjugate. Unconjugated dye was retained at the top of the column and the rapidly migrating zone was collected.

Adsorption of the conjugate with bovine liver powder was done according to the methods described by Coons and Kaplan.

Infected and control coverslips were removed from Leighton tubes and washed in phosphate buffered saline (ph 7.2) for five minutes. The coverslips were allowed to dry at 37 C, and fixed with acetone for ten minutes. Drying was again carried on for 30 minutes. At this time conjugate was applied to coverslips within a moist chamber and allowed to

*Pharmacia, Rochester, Minnesota.*
incubate at 37 C for one hour. The coverslips were washed with three changes of buffer and two changes of distilled water, and mounted in a 1:1 buffer (pH 7.2) glycerine mixture.

Tissues for fluorescent antibody staining were rapidly frozen by immersion in 2-methyl butane* kept cold by liquid nitrogen. Sections were cut at four microns using a Cryostat at -20 C. An alternative procedure was that of Sainte-Marie21 in which the tissues were fixed in 95 percent cold ethanol, dehydrated and cleared in cold absolute ethanol and cold xylene respectively and embedded in paraffin at 52 C. The blocks were stored at 4 C until sectioning.

Two sections from each tissue were pretreated with normal unlabeled gammaglobulin prepared in an identical manner as the specific antiviral gammaglobulin. The other section was pretreated with unlabeled antiviral gammaglobulin. Both slides were washed in phosphate buffered saline (pH 7.2) for five minutes with at least two changes of buffer. Both slides were then treated with the conjugate in a moist chamber at 37 C for 30 minutes. The sections were washed again in buffer and counterstained with Eriochrome black T,** prepared by the procedure of Hall and Hansen.7 Counterstain was not always used. The excess stain was removed by washing and sections were mounted in buffered glycerine.

The slides were examined by using a Leitz Ortholuz microscope with an Osram HBO 200 mercury vapour lamp Type L-2 and a 2 mm UGrl-UV filter. A dark field condenser was used in order to facilitate visualization of fluorescence.

Experimental calves. Eleven calves between four and eight months of age were used in transmission and pathogenesis studies. IBR and bovine viral diarrhea virus neutralization tests on preinoculation serum samples were negative. After the calves had been placed in the experimental units, a seven day adjustment period was allowed. During this time body temperatures were recorded twice daily and total white cell counts were recorded once daily.

Virus reisolation. Recovered nasal and rectal swabs were placed in five ml of saline and centrifuged at 12,000 rpm for 30 minutes. The supernatant fluid was recovered and incubated with penicillin (10^3 u/ml) and streptomycin (10^3 mcg/ml) for 30 minutes. Primary bovine testicle cell monolayers were inoculated with 0.1 ml of the supernate and allowed to absorb for one hour prior to the addition of Hank's lactalbumin medium supplemented with two percent ovine serum. Cultures were observed daily for cytopathic effects. Reisolation trials were considered negative after four passages at four day intervals.

RESULTS

Isolation of an IBR strain. The calf which had died with clinical signs of BVD-MD also had intestinal lesions characteristic of the disease.20 Most evident changes were those involving the Peyer's patches. Recovery

**Nutritional Biochemicals Corporation, Cleveland, Ohio.
of tissue for virus isolation was done by searing the serosal surface adjacent to a necrotic patch in the anterior ilium. After dissecting the seared serosa from the underlying mucosa, a sterile scalpel was employed to recover tissue debris from the deeper layers of the mucosa. It seemed that this method of specimen recovery would enhance the probability of recovering lesion associated agents rather than casual intestinal inhabitants. The tissue scrapings were then ground in a sterile Tenbroek grinder, diluted in four ml of saline G containing penicillin (10³ u/ml) and streptomycin (10³ mcg/ml). Centrifugation at 3000 rpm was carried on for 20 minutes after which time two ml of the supernate was transferred to a sterile tube and allowed to stand at room temperature for one hour. Calf testicle cell monolayers were then inoculated with 0.2 ml of the supernate and allowed to adsorb for one hour. After two - three day passages, cytopathic effects were evident.

*Strain purification.* In order to select a clone of the isolate, a plaque trial was attempted as previously outlined. In four days, plaques between two to four mm in diameter were observed. Three individual plaques were picked from three plates which had received one ml of a 10⁻⁶ dilution of second passage material. The agar was cut into a small square directly over the plaque area and immediately ground in a Tenbroek grinder containing three ml of saline. The saline was supplemented with 20 percent horse serum in order to prevent excessive loss of titer on freezing. A second plaque trial was made using one agar block from the first plaque selection as inoculum. In four days, a second plaque selection was done followed by the inoculation of several of calf testicle cell monolayers nurtured with Eagle’s basal medium unsupplemented with serum. After 36 hours, supernatant fluid was collected, centrifuged at 2000 rpm for 20 minutes, dispensed into five dram vials and stored at -70 C. Plaque titration indicated a titer of 10 PFU per ml. Three rabbits were hyperimmunized as previously described. Pre and post hyperimmunization serum from one rabbit was tested in a standard beta type neutralization test against 100 PFU's of the IBR strain and a strain of BVD-MD-Oregon C-24-V. The rabbit hyperimmune serum failed to neutralize the BVD-MD strain but neutralized the IBR strain completely at a 1:250 final dilution. It was concluded that plaque selection succeeded in establishing a clone of IBR hereafter designated as Iowa State University strain 1 (IBR-ISU-1).

*Fluorescent antibody tracing studies in cell cultures.* Fluorescent antibody tracing studies of intracellular viral antigens were done by infecting testicle cell monolayers on 22 x 11 mm no. 1 thickness coverslips, and harvesting both infected and control cultures at hourly intervals up to 22 hours. The cultures were inoculated with approximately 10⁶ PFU of IBR-ISU-1 resulting in an input virus-cell multiplicity of 10. Virus adsorption was carried on for one hour at which time the cells were washed three times, and reincubated with fresh medium. Fluorescent cell counts indicated that at least 90 percent of the cells were infected during this adsorption period.

As soon as 15 minutes after adsorption had begun, a diffuse fluorescence was seen over the infected cell monolayer which was absent in
control cultures. This was interpreted to be surface adsorbed virus antigen. At one hour, this diffuse fluorescence was not present.

At 90 minutes, the earliest specific developmental fluorescence was noted in the region of the nuclear envelope. This early fluorescence was always focal involving not more than one-tenth of the circumference of the nucleus. Between two and three hours, the nuclear membrane associated fluorescence extended and intensified. At this time, fluorescent blebs or vesicles could be seen at the nuclear surface. At four hours postinfection these blebs appeared as distinct fluorescent foci within the cytoplasm (Fig. 1). As infection progressed the character of the fluorescence gradually changed from the vesicular pattern, to more intense polar fluorescence, which spread completely throughout the cytoplasm. During the first 12 hours of infection nuclear fluorescence was not apparent except at the nuclear membrane. Even at 12 hours the lack of nuclear fluorescence was apparent (Fig. 2). At 23 hours the cytoplasm of the cells was intensely fluorescent. The apparent nuclear fluorescence at these later stages was thought to be superimposed cytoplasmic located fluorescence.

Many cells between seven and 15 hours were connected by fluorescent strands which often bridged infected with uninfected cells. More often than
not, the end of the strand in the uninfected cell was terminated by a fluorescent bead. (Fig. 2)

The specificity of the conjugate for virus antigen was demonstrated by the absence of fluorescence in uninfected cultures, by the gradual increase of the extent and intensity of fluorescence with time, and by the blocking of reactive sites using unlabeled anit IBR serum prior to staining with the conjugate. Two other strains of IBR (California-5 and Cooper) were labeled specifically with the conjugate with results identical to the IBR-ISU-1 strain.

**Serological characterization.** Plaque purified IBR-ISU-1, IBR-La, and IBR-Co, were used to hyperimmunize rabbits as previously described. Reciprocal cross virus neutralization was measured by plaque reduction tests. Virus neutralization titers were recorded as the reciprocal of the final serum dilution which neutralized 100 percent of the plaques. From the results presented in Table I, it is apparent that strains IBR-ISU-1 and IBR-La are identical serologically, whereas the IBR-ISU-1 strain and the IBR-Co strain appear to be nonreciprocally related.

### TABLE I

Reciprocal Cross Neutralization Study Between Strains of IBR

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Virus</th>
<th>IBR-ISU-1</th>
<th>IBR-Co</th>
<th>IBR-La</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR-ISU-1</td>
<td>250</td>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>IBR-Co</td>
<td>250</td>
<td>50</td>
<td>N.D.*</td>
<td></td>
</tr>
<tr>
<td>IBR-La</td>
<td>250</td>
<td>N.D.*</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

*Not done.

**Calf inoculation studies.** In order to evaluate the clinical response of calves receiving IBR-ISU-1, four animals were selected to receive intravenous inoculations of approximately $10^3$ PFU of cloned virus. Temperature recordings were made twice a day and white blood cells were counted once daily.

All four calves responded with a biphasic temperature elevation with the first temperature peak occurring between 24 and 48 hours after inoculation. In three of the four calves inoculated, leucoponia was present at this time. A second temperature peak was noted in all four calves. The time of onset of the secondary peak varied between the animals inoculated. The second peak was recorded at two days after the first peak in two animals and three and five days after the first temperature peak in the other two calves.

All of the calves went through a respiratory syndrome characterized by hyperpnea, forced abdominal breathing, and nasal discharge. The onset of these symptoms was noted at three days after inoculation in three animals and eight days after inoculation in one animal. In this later animal, the respiratory involvement had subsided in two days whereas in the other three calves respiratory symptoms were noted for seven to eight days.
Two of the four calves developed a distinct diarrhea. In both cases the onset occurred at three days post inoculation and persisted for approximately 10 days. During this time a gradual change in fecal consistency occurred characterized by a watery diarrhea at the onset, to a viscus mucous laden stool as the course of the diarrhea waned.

Seven calves between four and six months of age were selected for comparative pathogenesis studies. Three calves (nos. 2, 5, 15) were infected by injecting five ml of infected tissue culture fluid (10^7 PFU/ml) by the intravenous route. Two other calves (nos. 11 and 12) were inoculated intranasally with five ml of virus. The remaining two calves (nos. 7 and 14) were infected by being given five ml of tissue culture fluid by the oral route.

Since these animals were sacrificed relatively early in the course of the experimental infection, meaningful comparisons of clinical signs could not be made. Inoculated calves were sacrificed with subsequent tissue recovery at the times indicated in Table II. At this time tissues were processed for both histopathological and fluorescent antibody tracing studies.

No gross lesions were found in the oral cavity or in the esophagus of the inoculated calves. Gross erosions of the pillars of the rumen were present in calf 15. Abomasal folds were edematous in all animals. Ulcers in the abomasum were present only in animals infected by the intravenous route. Lesions in the small intestine were characterized by catarrhal enteritis of varying degrees with the most severe involvement occurring in the duodenum and ileum. The Peyer's patches were edematous and circumscribed with a hemorrhagic border; and on incising these areas, small necrotic foci were visible. Lesions of the large intestine were not consistent; however distinct fibrinous casts were attached to the colonic mucosa in calf 7.

Respiratory tract lesions were characterized by mild to moderate catarrhal rhinitis and tracheitis. Petechial to ecchymotic hemorrhages were present on the turbinates and walls of the frontal sinuses in calves infected by the intravenous and intranasal routes.

Gross enlargement of most of the lymph nodes was noted. Distinct hemorrhagic lesions measuring approximately one to two mm were present in the adrenal cortex of calves 5 and 15.

The most prominent microscopic lesions observed were associated with the gastrointestinal tract and associated lymph nodes. The lesion in the rumen in calf 15 was that of an acute focal necrotic and ulcerative rumenitis. The base of the ulcer contained cellular debris and abundant eosinophils. Hypertrophy, hyperplasia and hydropic degeneration of the stratified squamous epithelium of the rumen pillars were observed in calf 12. Abomasal lesions consisted of edema and mononuclear cell infiltration of the lamina propria. Lesions varying from lymphoid depletion to focal cystic necrosis of Peyer's patches were present in the small intestine. The necrotic lesions were more numerous in those animals infected by the intravenous or oral routes.

Depletion and necrosis of the lymphoid follicles were evident in the spleen and lymph nodes associated with the gastrointestinal tract and
TABLE II
Distribution of IBRSISU-1 Antigen in Tissues of Calves Following Inoculation

<table>
<thead>
<tr>
<th>Tissue Examined</th>
<th>Intravenous</th>
<th>Oral</th>
<th>Intranasal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days postinoculation</td>
<td>Days postinoculation</td>
<td>Days postinoculation</td>
</tr>
<tr>
<td></td>
<td>3 Calf 2</td>
<td>5 Calf 5</td>
<td>9 Calf 15</td>
</tr>
<tr>
<td>Tongue</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Esophagus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rumen</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Abomasum</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Duodenum</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Jejunum</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ileum</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cecum</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ileoceccocolic</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>valve</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Colon</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rectum</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Turbinate</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trachea</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bronchial lymph node</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescapular lymph node</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Retropharyngeal lymph node</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tonsil</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Epiglottis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adrenal</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spleen</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Myocardium</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Antigen detected
- Antigen not detected

respiratory tract. In some cases these lesions were very pronounced resulting in a pink staining sponge-like stroma.

Marked focal necrosis of the zona fasciculata containing an abundance of macrophage and necrotic cell debris was noted in the adrenal gland of calves 5 and 15.

Marked plasma cell infiltration of the turbinate was observed in all animals. The changes in the lungs were those of a mild interstitial pneumonia.

The distribution pattern of viral antigen in recovered tissues is presented in Table II. It is obvious that the agent was capable of generalizing
especially in regard to the intravenous and oral routes of the gastrointestinal tract, respiratory tract, and lymph nodes draining these areas. Fluorescent foci were noted in the tonsils of calves receiving virus intravenously and orally. In the lymphoid areas of the body, such as the Peyer's patches, lymph nodes, tonsils and spleen, the viral antigen was present in the necrotic or depleted germinal centers. In the spleen, however, the viral antigen was detected in higher concentration in the cells at the periphery of the splenic corpuscles.

In general, areas showing histopathological changes were always associated with positive fluorescence; however all fluorescent positive areas were not associated with histopathological changes. Representative fluorescent antibody stained tissue sections of turbinate epithelium, spleen and adrenal recovered from experimental calves are presented in Figures 3, 4, and 5 respectively.

A summary of virus reisolation attempts is presented in Table III.

DISCUSSION

The calf from which the IBR-ISU-1 strain was isolated came from a closed herd in Latimer, Iowa. In 1957 an outbreak of BVD-MD occurred in this herd when over a period of six months 21 of 23 cattle nine to 14 months of age died from this disease. Further losses from this disease did not occur in this herd until December of 1961. At this time 10 of 36 animals died from this disease over a period of six months. Malmquist

Figure 3. Section of turbinate epithelium showing infected epithelial cells (left) and exudate containing infected cells (arrow).
Figure 4. Section through spleen showing scattered infected cells.

Figure 5. Section through an adrenal abscess. Arrow indicates the limit of the abscess (left).
### TABLE III
Results of Virus Reisolation Attempts from Calves Inoculated with IBR-ISU-1 by Various Routes

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Route of Inoculation</th>
<th>Days post inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample</td>
</tr>
<tr>
<td>2</td>
<td>intravenous</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td>5</td>
<td>intravenous</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td>15</td>
<td>intravenous</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td>11</td>
<td>intranasal</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td>12</td>
<td>intranasal</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td>7</td>
<td>oral</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td>14</td>
<td>oral</td>
<td>nasal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rectal</td>
</tr>
</tbody>
</table>

+ Virus isolated  
- Virus not isolated

and Gutekunst* in a study of selected animals from this herd were able to isolate noncytopathic BVD-MD viruses in buffy coat cells of three calves over a period of three months. They also demonstrated that these persistent carriers did not develop BVD-MD serum neutralizing antibodies. The IBR strain isolated from this herd poses an interesting problem concerning its pathogenicity. In a serum neutralization study of calves during the 1961-62 episode using IBR-ISU-1 as the test virus, two of 10 animals tested showed titers of 1:4. That the two strains were isolated from one individual calf does suggest interesting biological relationships. Since viruses of both the BVD-MD and IBR groups demonstrate some lymphotropic behavior, the possibility of an additive effect of dual infection exists. Unpublished experiments of work done in our laboratory have demonstrated that noncytopathic BVD-MD virus propagated in bovine testicle cells will not interfere with the growth of IBR strains. However, an accentuating effect at the cellular level in vitro would be extremely difficult to measure in the light of the extremely rapid CPE shown by IBR strains. Tyler 25 has studied mixed infections of BVD-MD (Saunders strain) and IBR (Colorado strain) in calves. Calves doubly infected reacted more severely than when either agent was administered singly. Double infections always resulted in the production of a diarrhea while

*Personal communication, Ibid. Dr. D. Gutekunst, Jen-Sal Laboratories, Kansas City, Missouri.
single infections with the BVD-MD strain were inconsistent in producing diarrhea. Moreover, the severity and prolongation of the diarrhea in double infections suggested that an additive effect was operating.

Since both viruses exhibit some lymphotrophism, a possible additive effect could be explained by a lack of delay of antibody production on a local level to one or both agents. In fatal cases of BVD-MD where persistent viremia occurs, the absence of specific neutralizing antibodies suggests immunological tolerance to this virus. It is interesting to speculate from an immunological viewpoint on the possible effect of the introduction of a second virus during this period.

Serological comparisons with other strains indicate that the IBR-ISU-1 strain is identical to the Los Angeles (California-5) strain but non-reciprocally related to the Colorado (Cooper) strain. These results concur with those of Segre who demonstrated a one-way cross reaction between the Colorado (Cooper) strain and the Los Angeles (California-5) strain. It is difficult to speculate what these antigenic differences mean in the absence of careful pathogenesis studies with various strains of IBR.

We are familiar with two reports where a virus was isolated from cases of "mucosal disease" which in the absence of immediate serological studies were thought to be agents belonging to the BVD-MD group. Later unpublished reports identified these agents as belonging to the IBR group. In one of the cases cited above inoculation into experimental calves elicited a response like that of experimental viral diarrhea. This could represent an "unbiased" estimate of the pathogenic capability of IBR in the event that the IBR strain was uncontaminated by a BVD-MD agent.

On the basis of clinical and hematological observations and gross and microscopic lesions, it is apparent that the IBR-ISU-1 agent can produce a condition similar to experimental bovine virus diarrhea-mucosal disease. However, in one calf in which the virus was given intravenously and necropsied on the ninth day post inoculation, the lesions in the rumen and small intestine were more severe and resembled those described by Ramsey in field cases of mucosal disease.

In the staining of tissue sections with conjugate one of the major problems encountered is non-specific fluorescence. Two types of non-specific fluorescence are observed. One of these is the non-specific attachment of dye to tissue components, and the other is autofluorescence. The latter is the major limitation in detection of specific fluorescence in tissues such as the gastrointestinal wall. This is probably due to the extreme complexity of tissue, cellular, and subcellular elements in the wall of the gastrointestinal tract. In this study the use of the counterstain Eriochrome black T was found to be very effective in reducing the autofluorescence to a minimum without notably affecting the specific fluorescence.

The paraffin embedding technique of Sainte-Marie was found to be very useful because of better preservation of tissue and cellular architecture. However, frozen sectioning was generally preferred because of the shorter time required to process tissues.
These studies would indicate that the distribution of viral antigen in tissues as detected by fluorescent antibody tracing could be attributed to primary localization, multiplication and subsequent generalization from the primary site.

When the virus was administered by the intravenous route there was a rapid distribution throughout the entire body. Fluorescent antibody analysis indicated that there are some specific sites of localization and multiplication, namely the spleen, tonsils, Peyer's patches, and probably other lymphoid areas of the body. The extent of microscopic lesions varied greatly in these sites which could be attributed to differences in viral multiplication possibly because of differential cell susceptibilities or to differences in local immune mechanisms. Although attempts to detect virus in the turbinate epithelium by fluorescent antibody analysis were successful in only one animal of this group, virus reisolation attempts were successful in all cases (Table III). Since nasal scrapings were not recovered it is felt that the absence of specific fluorescence in the turbinate epithelium can be explained by the absence of infected cells at the section levels examined. An alternative hypotheses is that virus recovered by reisolation originated from infected tonsils or sinuses.

Persistence of viral antigen was noted in some tissues such as the spleen, tonsil, adrenal, bronchial lymph node and rumen even after the appearance of virus neutralizing antibodies. These sites of persistent fluorescence may represent intracellular live or inactivated virus which is inaccessible to antibody. It has been previously shown with the IBR-ISU-1 agent that giant cell formation occurs quite readily in cell cultures of bovine kidney but less readily in cultures of calf testicles. This difference may account for the persistence of virus antigen in some tissues, since polykaryocyte formation would serve to protect the virus from antibody and also promote local tissue extension.

Two animals infected intranasally were sacrificed on the fifth and seventh days post inoculation. One animal, sacrificed on day five, showed areas of specific fluorescence in the lung, trachea, spleen and mesenteric lymph nodes. A second animal, sacrificed on day seven, had fluorescent foci only in the turbinate. It was obvious that the virus is capable of generalizing when inoculated intranasally. However, the initial site of replication could not be determined from this limited study. In this study viral antigen was not detected in the kidney or the central nervous system.

Oral inoculation of virus in two calves resulted in fluorescent tonsilar foci at both the fifth and seventh day post inoculation. Other centers of infection were detected in the spleen, lungs, turbinates, and the gastrointestinal tract. Again it was apparent that the virus is able to generalize.

Despite negative blood cultures at all stages of the disease it seems reasonable to assume that virus extension from primary lymphatic sites is by way of infected cells of the lymphoid series. Negative blood cultures could possibly be explained by the observed leucopenia and increased chemotaxis which would lower the number of infected cells in circulation at any one time.
From these studies a pathogenesis scheme can be constructed. The primary focus of viral replication is at a lymphatic site most probably the tonsil. Generalization is effected by the subsequent distribution of infected white cells throughout the body via the thoracic duct. Direct extension of replicated virus from the tonsil to the nasal sinuses, or posteriorly to the trachea and esophagus cannot be ruled out. However, since intravenous inoculation of virus resulted in an enteritis, it is assumed that extension from the blood to gastrointestinal sites is possible. If the virus can replicate in the gastrointestinal tract as demonstrated by this work, the possibility of the direct extension to the vulva must be considered. Many vulvar lesions observed with IPV begin in close proximity to the central commisure of the vulva. The lamina propria of this region contains numerous lymph nodules which may serve as a source of susceptible cells for replication of virus originating from the gastrointestinal tract. An alternative hypothesis which seems to be supported by the herd study discussed below is that animals afflicted with IPV actually undergo a generalized infection although signs characteristic of classical IBR infections may not be readily observed. Lesions of IPV probably arise from generalized IBR infections, extension from the gastrointestinal tract, or possibly even by "muzzling" of the vulvar area by IBR infected cattle.

The use of the fluorescent antibody technique is suited for rapid diagnostic procedures involving tissue biopsied sections, nasal and vulvar scrapings. In a herd study involving 20 feeder cattle with genital lesions referable to infectious pustular vulvovaginitis, FA positive vaginal and nasal scrapings were recovered from four afflicted animals although overt signs of rhinitis were absent. A biopsy section of vulvar fold was processed for fluorescent antibody analysis. Fluorescing cells were limited to the lesion (Fig. 6) and fluorescence was not noted in a second section blocked with unconjugated antiserum. Nasal smears recovered from other animals revealed FA positive cells (Fig. 7).

SUMMARY

A strain of Infectious Bovine Rhinotracheitis virus (IBR) was isolated from a Peyer's patch of a calf which had died with lesions of typical mucosal disease. A second virus agent, isolated from the bone marrow of this animal was related to the bovine viral diarrhea-mucosal disease virus (BVD-MD). The IBR strain was isolated by plaque selection methods and shown to be closely related to the Los Angeles (California-5) strain of IBR by reciprocal plaque reduction tests. Inoculation trials were successful in reproducing a disease with clinical signs similar to experimental viral diarrhea.

Seven calves between four and six months of age were inoculated with the IBR-ISU-1 agent intravenously, intranasally or orally and were subsequently necropsied at varying intervals. Various tissues were collected for histopathological examination and fluorescent antibody tracing.

The portal of entry and initial site of virus replication apparently was the tonsil. In animals killed at later stages of the experimental disease,
the distribution of viral antigen was widespread throughout the body with a tendency to accumulate in lymphoid tissues. The virus was reisolated from the nasal discharge in most animals. However, it was thought that the rhinitis occurred subsequently to the generalized phase of the disease. Lesions of the gastrointestinal tract were similar to those of the bovine virus diarrhea-mucosal disease complex. Fluorescent antibody tracing studies of these areas revealed the presence of IBR-ISU-1 related antigen.

The fluorescent antibody tracing technique has been used successfully in the rapid diagnosis of field cases of infectious bovine rhinotracheitis and infectious pustular vulvovaginitis.

REFERENCES


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 as VS. This year, as in 1964, the Indiana type of the virus predominated with 273 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 altered 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, Greene 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 on 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.
BIRDS, VECTORS AND HOSTS SUSCEPTIBLE TO EASTERN AND WESTERN ENCEPHALITIS VIRUSES AND RELATED EPIZOOTIOLOGICAL ASPECTS: A REVIEW

David C. Tudor, V.M.D.

Biologically, a broad host spectrum increases the survival probability of any arthropod-borne disease. Encephalitis is a problem of considerable concern in the United States. The source of vector infection can be found in a wide variety of domestic and wild birds and animals.

The earliest reports of the disease concerned horses.29,42,90,141,255,257, 274,282,285,292,376,383,384 Campbell 42 reported the presence of the disease in horses in Wertenberg, Germany, in 1813, and in the United States in 1847. Others29,90,255 also reported the disease in the United States in 1847, but earlier reports141,282,285,376 suggested its presence here in 1831. In 1909, Dr. Feemster 87 reported that the disease existed in horses in Minnesota. In 1912, encephalitis in horses was reported as prevalent in Kansas, Nebraska, Colorado, Oklahoma, and Missouri from early August to the advent of frost in September and October; 35,000 horses died during this outbreak of the disease.384

Two types of the encephalitis virus have been isolated, the Western type (WE) and the Eastern type (EE). Moussu and Marchand271a and Zwick and Seifried43 working in Europe in 1924 determined that equine encephalitis, Western type, was due to a filterable virus. In the United States WE virus, found primarily west of the Mississippi, was isolated in 1930 by Meyer, Haring, and Howitt in California.257 These investigators reported a mortality of 3,000 horses from 1915-1920 in California, Colorado, Oregon, Nevada, and Montana. Later, in 1932, Meyer 254 suggested the possibility of human infection following WE infection in horses, and he was the first to describe human cases.

In 1933, Giltner and Shahan103a investigated an epizootic in horses in New Jersey, Virginia, Delaware, and Maryland, and isolated a virus that was shown to be distinctly different from the WE virus. In the same year, TenBroeck, Hurst and Traub369 also recovered EE type virus from infected horses from the same area. This EE virus has since been found along the eastern seaboard and the Gulf of Mexico and has been isolated from many different species including man. In 1938, Fothergill et al.94 and Webster and Wright391 in Massachusetts were the first to report human infection with this EE virus.

In 1938, Beaudette sent a pheasant specimen that had died of encephalitis to Van Roekel387 in Massachusetts. This was the first isolation of EE virus from pheasants. The first case of this disease in pheasants to be reported occurred later that year in Connecticut, where an outbreak of the disease in pheasants was investigated.

In the same year (1938), in Massachusetts, Fothergill and Dingle re-
covered EE virus from the brain of a pigeon\textsuperscript{93} that had been bred on a farm adjacent to another farm where two horses had died of the disease. This was the first evidence of a possible relationship between birds and the disease in horses.

It was not until 1941, however, that WE virus was recovered, by Cox and his associates,\textsuperscript{62} from a bird, a Prairie chicken, in Regly, North Dakota. Later (1953), WE virus was also isolated from English sparrows in New Jersey.\textsuperscript{164}

The presence of antibody to EE virus in the serum of domestic birds was first reported by Tenbroeck,\textsuperscript{368} and subsequently by other authors.\textsuperscript{182,183} Serological surveys indicate that to date no species of bird has been found to be refractory to infection with one or both viruses. Such surveys provide an estimate of the extent of the disease. A positive finding indicates that the specimen has been infected, but a negative result does not prove that the individual did not or could not contract the infection. It is conceivable that individual specimens could also lose their antibody titer. It should be noted, however, that EE virus concentrations in the blood of small passerines reaches much higher levels than in larger species, such as herons and ibis.\textsuperscript{224,259} Antibody titers may also vary, depending upon the stage of the infection when blood is secured and the degree of antibody production by the individual. Mammals also have different degrees of susceptibility varying from refractory to highly susceptible.\textsuperscript{222,317}

The virus of WE was first found in mosquitoes in 1941,\textsuperscript{132,134} but in 1933 Kelser,\textsuperscript{212-215} working on the transmission of the disease by mosquitoes in Panama, had been the first to show that mosquitoes, and specifically \textit{Aedes aegypti} and \textit{Aedes taeniorhynchus} mosquitoes, could transmit the WE virus from guinea pig to guinea pig, and in 1938 Howitt\textsuperscript{176} was the first to report the isolation of WE from a child with encephalitis to the San Joaquin Valley in California.

Continued study of the disease has resulted in numerous reports of isolations, antibody determinations, and host susceptibility. Table I, a survey list of such reports, includes mammals, birds, mosquitoes, lice, mites, fleas, flies, black flies, assassin bugs, ticks, snakes, alligators, turtles, lizards, frogs, and fish. Over 25 species of mosquitoes have been found capable of transmitting the virus in the laboratory, and virus has been recovered from over 30 species. \textit{Culexita melanur} has been related by many investigators\textsuperscript{32,33,76,149,167,168,198-202} to outbreaks in birds. It has also been demonstrated that \textit{Culexita melanur} feeds predominantly on such birds as quail and pheasant, and on other small wild birds, but it is reluctant to feed on man.\textsuperscript{32,33,88} \textit{Culex tarsalis},\textsuperscript{1,18,293,298} \textit{Culex salinarius},\textsuperscript{32,57} \textit{Culex restuans},\textsuperscript{196,198} \textit{Aedes sollicitans}\textsuperscript{32,53,149,197,198,201} are among other species\textsuperscript{149} that have been associated with human outbreaks. Hayes \textit{et al.},\textsuperscript{149} however, considers \textit{Aedes vexans} as the chief vector in the development of the disease in horses.

In any particular epidemic, such as the one New Jersey experienced in 1959\textsuperscript{76,149,197-199} when 21 out of 33 confirmed human cases of EE died, it is very important for public health workers to understand the nature of the agents involved and which vectors and which host animals are instru-
mental in maintaining the epidemic. It is essential to determine the relative vector efficiencies and also vector-host-virus relationships. In such an arthropod-borne-arbovirus disease it is necessary to know which vectors are potentially the most efficient as well as which animals are capable of infecting them. Many kinds of warm- and cold-blooded animals are susceptible to mosquito-borne infection, but only certain ones develop enough virus in their bloods to infect vector mosquitoes with minimal infection thresholds.

To be an effective carrier reservoir of infection, the host must be susceptible to infection and must circulate a threshold level of virus sufficient to infect the vector. The virus must survive and multiply within the host if sufficient concentration of blood virus is to be available for vector transmission. Some hosts circulate lesser concentrations of virus and are able to infect only those mosquitoes with the lowest infection thresholds. Other animals develop so little virus that they only are rarely capable of infecting even the most susceptible mosquitoes. The virus must also be present in the blood of the host producing a viremia for a sufficient length of time to permit vector infection, otherwise vector feeding and thus transmission is limited. In horses this viremic period is short; in addition, the virus titer is ordinarily too low to serve as a satisfactory source of infection for mosquitoes. Horses are thus considered as blind hosts for virus transmission.

In an effort to evaluate vector efficiencies, Chamberlain listed three basic criteria: "threshold of infection," "infection rate," and "transmission rate." He defines the "threshold of infection" as the lowest concentration of virus which will infect at least a minimal percentage (between 1 and 5 percent) of mosquitoes; a considerable amount of virus is required to infect some species of mosquitoes. The mere recovery of virus from an engorged vector does not necessarily incriminate the anthropod as a transmitter of the disease, since it may have recently ingested the virus in a blood meal or be incapable of transmitting the virus to a vertebrate host. The virus must not only survive in the vector, possibly for life, but must multiply after a suitable extrinsic incubation period for the salivary glands of the vector to become infective and for biological, rather than mechanical, transmission to occur. In some vectors, such as ticks and possibly mites, transovarial transmission may occur.

"Infection rate," a more important criteria of efficiency, is defined by Chamberlain as the percentage of mosquitoes retaining virus in their bodies for at least one or two weeks after ingesting a standard high concentration of blood virus.

The "transmission rate" is the percentage of the mosquitoes which, after ingestion of the virus meal, are able to transmit infection to susceptible animals when permitted a single opportunity to refeed individually. This rate may be altered by temperature, and according to Reeves and Hammon a minimal threshold of sustained temperature is required for effective virus transmission of WE.

In evaluating vector transmission, selective feeding habits of mosquitoes must also be considered. Mosquitoes that feed predominantly on birds, such as Culex pipiens and Culiseta melanura, even though heavily
infected, would not be a potential hazard for mammals. On the other hand *Aedes sollicitans, Mansonia perturbans* and *Anopheles quadrimaculatus* are dominantly mammalian feeders and would not be expected to contribute extensively to bird-to-bird transmission.

The stability or "infectivity" of the virus is also a major concern in the vector-host virus relationship. Although, fortunately, EE virus is quite susceptible to unfavorable environmental conditions, it is relatively stable in the dried state, and this biological aspect could conceivably be a factor in its transmission.

Epidemics are not only dependent upon the relative efficiency of the vector or host in transmitting the disease, but are dependent upon a large concentrated population of highly infected hosts when susceptible vectors are plentiful, for instance after heavy rains or a flood tide. The reverse condition with an abundance of infected vectors and susceptible hosts must also occur.

In most instances, an epidemic can only develop if an endemic situation has been maintained. Serological surveys have shown inapparent infections to be very common in man, animals, and birds. In such surveys, high antibody titers in endemic areas are suggestive of stationary foci which help to maintain infection throughout the year. Ample evidence has been assembled to indicate that birds are the usual source of vector virus, but serological surveys have yielded considerable information concerning the ecology of the disease and have suggested that the source of spring vector infection does not entirely reside in resident or migrating bird populations. The overwintering virus that kindles the summer epidemic may be residing in hibernating mosquitoes, snakes, snakes, snakes, snakes, turtles, turtles, turtles, or rodents.

Recent work has provided further information in this area. It has been demonstrated that arbovirus was present in birds and mosquitoes in an area where no human transmission was detected. Wild birds, including residents, were found infected as early as June 12, one full month before any isolations in mosquitoes, and as late as December 14. Results of this and other work strongly suggest the possibility that some reservoir host is present the year round, and that in reality the infection may not be introduced by annual migratory birds from warmer climates, although antibody levels of southern migratory birds have been shown to be higher than those of northern resident birds.

In summary, the tabulation of the many virus isolations and antibody determinations indicates the extensive involvement and transmission of EE and WE within a broad host and vector spectrum. Encephalitis has become a problem in a wide variety of animals and birds, because of the great number of infective hosts, the extensive vector population, and the ability of the viruses to overwinter.

ACKNOWLEDGMENTS

The author gratefully acknowledges the valued assistance of Miss Joyce Dellecker, Miss Ruth Field, Mrs. Anna Stover and Mr. Howard Woodward.
### TABLE I
Species Naturally or Experimentally Susceptible to EE or WE
(For explanation of symbols, see end of table)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Common Name</th>
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<th>Western Encephalitis</th>
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<td><em>Sciurus Carolinensis</em></td>
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TABLE I (Cont'd.)

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<td>Kangaroo Rat</td>
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<td>Sigmodon hispidus</td>
<td>Cotton Rat</td>
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<td>Rheithrodontomys megalatus</td>
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<td>Peromyscus maniculatus</td>
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<td>Peromyscus truei</td>
<td>Mouse - Field</td>
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<tr>
<td>Peromyscus maniculatus</td>
<td>Mouse - Meadow</td>
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<tr>
<td>Microtus mordax</td>
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<tr>
<td>Microtus californicus</td>
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<td>Microtus montanus</td>
<td>Mouse - Field</td>
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<td>A123;180.</td>
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<td>Mouse - Swiss albino</td>
<td>268;269;382.</td>
<td>62;71.</td>
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<td>Mouse</td>
<td>2d;49;70;104;VR156;</td>
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<td>VR157;216;277;329;</td>
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<td>353;370;391.</td>
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<td>Microtus pennsylvanicus</td>
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<td>Vole - Meadow</td>
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<td>Cricetus sp.</td>
<td>Hamster - Syrian</td>
<td>389.</td>
<td>71;VR156;VR157;</td>
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<td>264a;389.</td>
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<td>Guinea pig</td>
<td>70;91a;173;175;277;</td>
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<td>307;327;329;370;379;391</td>
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<td>Guinea pig</td>
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<td>106*.</td>
<td>A123.</td>
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<td><em>Sylvilagus floridanus</em></td>
<td>Rabbit -</td>
<td>A152;364.</td>
<td>VR108;364.</td>
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<td><em>Sylvilagus audubonii</em></td>
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<td><em>Sylvilagus bachmani</em></td>
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<td>brush</td>
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<td>A123;A188;364.</td>
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<td><em>Lepus townsendii</em></td>
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<td><em>Lasius cinereus</em></td>
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<td>Bat - small grey</td>
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<td>Alligator</td>
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<td><em>Hyla crucifer</em></td>
<td>Spring peeper -</td>
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<td>VR108;VR66.</td>
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<td><em>Rana pipiens</em></td>
<td>Northern</td>
<td>A148;152.</td>
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<td><em>Opheodrys aestivus</em></td>
<td>Rough green</td>
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<td><em>Elaphe guttata</em></td>
<td>Red Rat</td>
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<td><em>Achistodon piscivorus</em></td>
<td>Cotton Mouth</td>
<td>Moccasin</td>
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<td>Eastern Hognose</td>
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<td>Natrix sipedon</td>
<td>Water</td>
<td>A148;152;205.</td>
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<td>Coluber constrictor</td>
<td>Black Racer</td>
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<td>Bothrops ammodytoides</td>
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<td>Bothrops alternata</td>
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<td>Garter (Eastern)</td>
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<td>Piterophis sp.</td>
<td>Pine Snake</td>
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<td>Eastern Box</td>
<td>A148;A152.</td>
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<tr>
<td>Malaclemys centrata</td>
<td>Diamond-backed</td>
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<tr>
<td>Chelydra serpentina</td>
<td>Spotted</td>
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<td>Snapping</td>
<td>A148;A152.</td>
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<td>Wood</td>
<td>A148;152.</td>
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<td>Chrysemys picta</td>
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<td>Gopherus polyphemus</td>
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<td>Sternotheraeus carinatus</td>
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<td>Trionyx ferox</td>
<td>Florida Softshell</td>
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<td>Terrapene carolina</td>
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<td>Turtle</td>
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<td><strong>Lizards:</strong></td>
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<td>Broad-headed Skink</td>
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<td><em>Anolis carolinensis</em></td>
<td>Green Anole</td>
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<td><em>Ophisaurus sp.</em></td>
<td>Glass</td>
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<td>VR108.</td>
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<td><strong>Fish:</strong></td>
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<td>Fish - Mosquito</td>
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<tr>
<td><em>Eumenocanthus stramineus</em></td>
<td>Chicken - body louse</td>
<td>2d;VR183.</td>
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<td><em>Menopon pallidum</em></td>
<td>Chicken - body louse</td>
<td>VR183.</td>
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<td><strong>Mites:</strong></td>
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<tr>
<td><em>Liponyssus bursa</em></td>
<td>Mite - feather</td>
<td>2d;VR357;VR358.</td>
<td>VR260;VR300.</td>
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<td><em>Liponyssus sylviarum</em></td>
<td>Mite - feather</td>
<td>2d;VR135;VR299; VR300;VR392a.</td>
<td>VR260;VR300.</td>
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<td><em>Demanyssus gallinae</em></td>
<td>Mite - red</td>
<td>VT2d;VR183;VR356.</td>
<td>VR260;VR300.</td>
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<td>Louse</td>
<td>VR108.</td>
<td>VR183.</td>
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<td><em>Eusimulium johanseni</em></td>
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<td>VR2a.</td>
<td>VR275.</td>
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<td><em>Simulium meridionale</em></td>
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<td><em>Dermacentor americanus</em></td>
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<td><em>Dermacentor andersoni</em></td>
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<td><em>Dermacentor variabilis</em></td>
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<td><em>Dermacentor marginatus</em></td>
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<td>304b.</td>
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<td>Argus persicus</td>
<td>Fowl - stick tight</td>
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<td>265;VT366.</td>
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<td>Rhipicephalus sanguineus</td>
<td>Brown Dog</td>
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<td>LeConte</td>
<td>VR2d;VR111;VR230;</td>
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* Not designated as EE or WE

**A Antibodies**

**R Recovered**

**T Transmitted**

**V Virus**

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5. Barnett, H. C.: Experimental Studies of Concurrent Infection of Canaries and the Mosquito *Culex tarsalis* with Plasmodium relictum and Western Equine


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SUSCEPTIBLE BIRDS, VECTORS AND HOSTS


RESEARCH FINDINGS IN EQUINE INFECTIOUS ANEMIA


I. Introduction

Equine infectious anemia (EIA) is an infectious viral disease of sol-peds in which it is generally believed that the "recovered horse" has a persistent viremia for the duration of its life. Reports from Texas, unconfirmed by experimental procedures, suggest that young animals do recover completely from FIA, their blood being free of virus. Because of the persistant viremia, the disease is disseminated mechanically by blood-sucking arthropods and by contaminated instruments. The experimentally infected animal may present one or all of three clinical syndromes: (1) Acute case - wherein the animal develops a continued high fever of 105°-107°F until death occurs five to 20 days following its inception. Anemia is mild or non-existent and the horse dies of a toxic syndrome, leukopenia, elevated serum lactic dehydrogenase (LDH) and elevated serum lipoproteins. Varying degrees of ventral edema may be present. Weakness is exemplified by the horse dragging its hind feet. (2) Chronic carrier - wherein the horse develops fever of varying lengths of time in days, the fever perhaps not being as high as in the acute case, but during which time the horse becomes anemic, the ventral parts edematous, and the animal again shows weakness indicated by dragging the hind feet. This chronic stage may persist for weeks, followed by death or by apparent recovery wherein the fever subsides, the anemia gradually disappears, and the animal resumes a nearly normal appearance. (3) The third type is the asymptomatic carrier. The asymptomatic carrier may follow the chronic carrier stage or it may follow the acute stage cases that do not die. The animal is afebrile and its blood values are essentially normal. The animal may be in good flesh without any sign of disease, although occasionally it will present a slight weakness evidenced by dragging the hind feet. At unpredicted intervals, from two weeks to one and one-half years, the animal may experience fever of four to eight days. Its appetite remains good. It may recover from the fever or it may develop fulminating disease and die.

The disease is worldwide in distribution, although some countries are reported free of it. A questionnaire sent in the summer of 1965 revealed that 26 of 48 states reported diagnosing the disease in the past five years. Persons interested in learning more of EIA may consult two general references on EIA, Dreguss & Lombard and Ishii. The Japanese have been very active in research in recent years and presumably
through their research efforts have reduced the infection rate from 0.8 percent in 1955 and 0.29 percent in 1964. Dr. R. W. Moore, Texas A & M, has been conducting a precipitin test for EIA; Comparisons between our known infected animals and his test have been 76-84 percent correct, but these tests were on frozen serums which supposedly do not give reliable results.

Although rather extensive research work has been done on the disease, a specific diagnostic test other than testing suspect material in a susceptible horse has not been developed. Recognizing that from a practical standpoint the most pressing problem was to devise an accurate diagnostic test to detect the carrier animal, but at the same time also recognizing the need to approach the study of the disease from a broad viewpoint using the newest of equipment and knowledge, we submitted an application to the National Institutes of Health for a research grant to obtain financial support. The grant application in 1961 proposed to study the disease from several broad aspects: (1) Physical and chemical nature of the infectious agent, (2) adaptation of the infectious agent to tissue culture or laboratory animal, (3) biochemical studies, including the use of isotopes, to further characterize the alterations in the infected animal, (4) histopathologic and electron microscopic studies of tissues from the affected horse, (5) applications of various serologic methods and skin tests to discover a method which might be specific for EIA. Our principal objective nevertheless was to develop a specific test to detect the asymptomatic carrier horse.

The basic tenet of this report, then, is to present in brief a summation of our research results on EIA; important aspects of the study, sufficiently complete to warrant so, are being published in detail elsewhere. This report, however, certainly does not, or cannot hope to, contain all the suggestions proposed in the grant application. Finally, since the grant has not been renewed, we shall not be able to complete in detail many of our original proposals; suggestions here, however, may provide the background for future investigations. A discussion of the pertinent research findings follows.

II. Biochemical Alterations in Serum

A. Altered Serum Lipoproteins; Albumin/Globulin Ratios:

As the horse sickens with EIA, its serum lipoproteins change quite abruptly. Normally, the serum lipoproteins range from 100 to 300 mg. percent; with progression in the disease, the values rise to ten times this value. With this rise in total lipoproteins, there is a drastic shift in the electrophoretic nature of these lipoproteins. The two electrophoretic fractions in normal horses have alpha fractions of 40 percent to 80 percent, mean of 57, standard deviation of 5.7 percent, or expressed as a ratio of alpha/total lipoprotein of 0.4 - 0.8 etc.; in EIA, the alpha fraction remains essentially the same while the beta fraction increases, accounting for altered percent alpha values of as much as ten-fold. Therefore, in EIA the altered serum lipoprotein may be expressed as a rise in total lipid (to 10 fold increase) or as an altered electrophoretic value.
(1/10 the normal value). The cause for the altered serum lipids may be
due to altered liver function and even direct extrusions of fat from body
stores into the circulation. We have found this test to be helpful in re-
cognizing the infected horse, but it must be remembered that toxic livers
from organic or inorganic poisonings may result in altered serum lipo-
proteins.

Albumin/globulin (A/G) ratios were done on experimental horses for
a long time. Although in acute cases these ratios do fall terminally to half
the pre-inoculum values, A/G's from animal to animal vary considerably
making use of the A/G ratio diagnostically rather precarious. In chronic
carriers of long duration, the A/G's may go to 0.1 to 0.2; advanced para-
sitism, state of nutrition, and age also affect these ratios.

B. Altered Serum Lactic Dehydrogenase:

Serum enzymes increase in infectious canine hepatitis, encephalo-
myocarditis of swine, and, of course, in many severe pathologic process-
es of man and animals. A study was done to check serum of horses with
EIA for the presence of altered serum enzymes. Doctor Kuhns, one of the
co-authors, is responsible for conducting these tests and for finding that
no alterations occur in serum SGOT and ICD in EIA but that there is
marked rise in lactic dehydrogenase (LDH) in the course of EIA. Norm-
al values expressed as Berger-Brodia units tested with Sigma Chemical
kit range from 320 to 830, mean of 572, SD of 148. In EIA, values
frequently reach 2000; in terminal cases values have gone as high as
19,800. The tissue origin of this altered serum LDH in EIA is unknown.
LDH is an enzyme present in the cell sap of many cells. We have postu-
lated that it rises in EIA as a result of the hepatitis which occurs; it is
further considered that the hepatitis is due to a toxic effect of the virus
upon the liver rather than to very much direct viral replication in the
hepatic cord cells. The delayed rise in LDH, beginning a few days after
the onset of fever, somewhat supports this contention. Red cell hemolysis
releases LDH; therefore, tests for LDH must be on serum completely free
of hemolysis.

III. Histopathology and Electron Microscopy

A. Histopathology:

Histopathologic findings in this study are restricted (1) to a discus-
sion of the hepatic changes since liver biopsies are used in an attempt to
recognize the infected horse and (2) to a vascular change which possibly
accounts for the altered lipemia.

(1) Liver biopsies from horses in the asymptomatic stage of the dis-
ease may appear normal. Whether or not death ensues and depending
somewhat upon the duration of the febrile period, the liver sections pre-
sent varying degrees of hemosiderosis, swelling of the Kupffer cells, and
lymphocytic infiltration of the sinusoids. There may or may not be

*Sigma Chemical Co., St. Louis, Mo.
centrolobular degeneration which, if present, is probably a manifestation of anoxia. In this study, equine babesiosis was found to be accompanied by essentially the same liver changes as in EIA. Roberts, however, failed to find lymphocytic infiltration into the hepatic sinusoids in equine piroplasmosis.

(2) The reason for the terminal lipemia that occurs in EIA is not known, but histologic examination of fat from one horse dying with Wyoming (W) strain infection presented the appearance of fat in the lumen of the capillaries. The entholial cells lining the fat depots were damaged, seemingly permitting the fat to be picked up directly by the circulating blood.

B. Acridine Orange Staining of Erythrocytes - Electron Microscopy - Myelin Sheath Structure:

The fluorochrome dye acridine orange is used quite extensively in our laboratory for demonstrating mammalian erythrocytic parasites. In the course of examining erythrocytes from horses with EIA, it was noticed that up to 50 percent of the cells just prior to death contained small particles on their surfaces. The particles were barely visible at 1000 magnifications. These particles were also seen, however, in terminal piroplasmosis, and their significance is not known except perhaps that they are degenerative processes accompanying the lipemia. At the electron microscopic level they resemble "myelin sheath structures," having an overall diameter of 62.5 -150 \mu, with a periodicity of 62.5 angstroms.

Electron microscopy of livers revealed a mosaic arrangement with virus-like particles 20 \mu in diameter; electron microscopy of kidneys revealed the presence of microbodies. The size of the particles in the liver cells does not fit filtration sizes of the virus, but the size of virus in the cell may be smaller than outside the cell if an additional protein coat is added to the virus as the virus is extruded from the cell.

IV. Serologic - Virologic - Skin Tests

A. Heterophile Antibody - Lack of Antibody to Lipovirus

Dr. Robert Chang in his studies with "lipovirus," which was isolated in tissue culture from a child with infectious hepatitis, reported four of nine horse serums to contain antibodies to the "lipovirus." In view of the hepatitis in EIA and its possible connection with infectious hepatitis or serum hepatitis of man, and its widespread distribution in nature, it was considered desirable to test various EIA serums against this "lipovirus." Therefore, several preinoculum and postinoculum (p.i.) serums from horses artificially infected with the Wyoming strain of virus and from a donkey artificially infected with a Florida (F) strain of virus were sent to Dr. R. Chang for testing. All of the serums, however, contained no "sensitized erythrocyte agglutinin" antibodies to "lipovirus." Several isolation attempts on acute phase EIA heparinized bloods or serums utilizing conditions optimal for the multiplication of the lipovirus yielded no transmissible agent. The complement fixation test was complicated by the fact that most of the horses developed a rise in antibody to normal human tissue
following infection with EIA virus; this rise in antibody against human tissue (e.g., as high as 1/32 twelve days after inoculation in one horse) is a very impressive and consistent finding but probably just represents heterogenetic antibody response. This finding further supports the nature of a heterophile antibody response against chicken erythrocites as described by Dreguss in 1949 and in part the autologous antibody (anti-antibody) against erythrocytes as demonstrated by the positive direct Coomb's test described in the next paragraph.

B. Direct Coomb's Test - Autoantibody:

The increased erythrocyte sedimentation rate in the febrile stages of equine infectious anemia may be expressed in part by the presence of autoantibody; support for this thesis may be demonstrated by washing the erythrocytes with saline and then testing a dilute preparation of these cells with anti-equine globulin serum, rabbit origin.* Erythrocytes from horses in the severe febrile stages of EIA are agglutinated by the anti-equine globulin serum, demonstrating the presence of globulin on the washed erythrocytes. This positive direct Coomb's test becomes negative in the asymptomatic carrier horse, however. The reason for the positive direct Coomb's test is not known but is postulated that the EIA virus is intimately attached to the erythrocyte and antibody is formed against this complex; the globulin attached cannot be easily dissociated.

C. Hemolysins from Livers of Infected Animals:

In searching for clues that may suggest tissues rich in EIA virus, 20 percent suspensions of livers, spleens, and fat of normal or non-EIA animals and of animals that had died from EIA were emulsified for two five minute cycles at 45,000 r.p.m. in a Virtis** homogenizer. The container was kept in crushed ice. (Parenthetically it should be mentioned that serums from the acute stages of EIA, when differentially centrifuged at 10,000 r.p.m. for 20 minutes followed by 40,000 r.p.m. for two hours, generally failed to demonstrate a pellicle that could be concentrated or would react in antigen-antibody systems such as complement-fixation and agar gel precipitation. A suggestion of a viral component was detected in terminal serums scanned vertically in quartz tubes in a DU spectrophotometer**** following their layering over successive increases in sucrose solutions of 11, 20, 29, 36 and 45 percent; 39,000 r.p.m., four to 12 hours, 2 - 10°C.)

Following Virtis treatment and differential centrifugation in the Spinco*** with a concentration of approximately ten fold from the original tissue suspension, washed equine erythrocytes were incubated with increasing dilutions of these tissue preparations. Preparations of livers

* Mann Research Laboratories, N.Y., N.Y.
** Virtis Co., Inc., Gardiner, N.Y.
**** Beckman Instruments, Fullerton, Calif.
RESEARCH FINDINGS IN EIA

from two EIA animals, one the F strain and one the W strain, hemolyzed equine erythrocytes to 1/32- and 1/640 respectively, a liver preparation from a normal horse was non-hemolytic. Preparations of spleen and fat from infected horses were essentially non-hemolytic. The hepatic lytic factor was readily sedimentable since 30,000 r.p.m. for 10 minutes removed the lytic factor from the supernatant.

The hemolytic factor from the livers was not inactivated by heating to 56° for one hour. Serums from EIA carrier horses, when incubated with four - 40 units of this hemolysin, lacked anti-hemolysins, although both normal horse serums and EIA carrier serums at 1/5, 1/10, and 1/20 dilution prevented hemolysis, presumably due to the large amount of serum proteins present.

The liver hemolysin preparations, when examined in the sucrose gradient ultracentrifuge systems, produced a strong band 3/4 of the way down the tube following centrifugation at 39,000 for 15 hours. The F strain material was somewhat lower down the tube than the W strain.

Collection of the various fractions illustrated that most of the hemolysin resided in the heavy lower centrifuge band. No such lower heavy band was present from the liver preparation of a horse dying from acute sand colic.

Subsequent study of this liver hemolysin was confusing, however, in that a liver preparation from another W strain infected horse did not contain hemolysins, whereas a liver preparation from a splenectomized horse dying with piroplasmosis contained the hemolysin. It was concluded that these hemolysins were not specific for equine infectious anemia, but perhaps were similar to increased levels of lysozyme activity in the liver which may occur in EIA or piroplasmosis due just to the toxic nature of the infectious agents or to chemical toxic injury to the liver.

D. Interactions Between EIA and Other Viruses:

Interference between closely related viruses or unrelated viruses has been established as a certainty. The persistent viremia which occurs in EIA suggested the hypothesis that an interference system or a synergistic system would provide evidence for quantitation of EIA virus in horse serum or plasma and might, moreover, provide a diagnostic test system as does the RIF method for avian lymphomatosis or the END method for hog cholera. Consequently, interacting systems were set up between normal horse serum and EIA serum and certain viruses, using mice or embryonating eggs as test systems. Interference was also tested in tissue cultures and is discussed in the section on tissue culture.

Early in our studies, when eastern, western, or St. Louis encephalitis viruses were used as challenge viruses, there appeared to be a slightly synergistic effect between the EIA virus and these agents. The LD₅₀ dose dilution in mice following the combination was often higher than the mice receiving normal horse serum plus challenge virus. The level of "synergism" was low, however—usually less than one log—and was probably not more than experimental error, even though the results were generally consistent and reproducible. A trial in embryonating eggs giving EIA or
normal horse serum followed by the PR8 strain of influenza* sixteen hours later, resulted in no difference in death times or numbers between controls and embryos receiving EIA virus. In a subsequent experiment in which western encephalitis virus was given to embryos after normal or acute EIA serum, there was evidence again of synergism between the two agents as seen by slightly enhanced LD₅₀'s.

Many subsequent experiments using the normal pre-inoculum serum or plasma as a control followed by acute, asymptomatic, or terminal sera or plasmas in mice challenged with the encephalomyocarditis (EMC) virus, resulted in the following general conclusions: (1) Terminal sera contained the most interfering activity and reduced the LD₅₀ of EMC virus for mice by as much as 10²⁷⁵ logs of virus; using lesser levels of challenge and expressing endpoints as survival times, the survival indices would attain values as great as 4.63. (2) Higher interfering activity occurred when EIA was given three hours before EMC than at 38 minutes, 6-1/3, 9-1/2, 20, or 30 hours. (3) Several factors, as follows, had no appreciable effect upon the interfering properties: (a) .5 percent formalin, 37°-16 hrs., 4°-30 hrs.; (b) .5 percent Wescondyne -37°, 16 hrs., 4°-30 hrs.; (c) 50/50 with chloroform 37°, 16 hrs.; chloroform allowed to evaporate; (d) 37°-16 hrs. room temperature - 24 hrs.; (e) pH 5.0-37°, 18 hrs.; (f) 56°, one hr. (4) Leucocytes concentrated from the peripheral blood of chronic carriers, asymptomatic carriers, and normal horses contained, in decreasing order, low levels of interfering activity; e.g., in one experiment, leucocyte extract from a chronic carrier gave an interfering index of 1.33 logs and an asymptomatic carrier a level of .77 logs. Work along these lines is continuing.

**E. Tissue Culture:**

Dr. A. J. Kniazeff performed certain tissue culture aspects of this study. Acute phase serum of the W strain worked with widely in the Kissimmee study and splenic homogenate from the tenth day or so of a natural Florida infection were passed ten times on continuous equine thymus monolayers. Passages were made weekly; at each passage, the tissue culture cells and medium were sonicated for ninety seconds, diluted 1/10 and transferred to new thymus cells. The medium was Eagles MEM containing essential amino acids plus fetal calf serum known to be free of globulins.

Beginning with the fifth passage, the monolayers were exposed to possible interference to vaccinia virus. Using the plaque assay method, greater than a fifty percent reduction in plaques occurred with the cells presumably infected with the EIA virus; this fifty percent reduction is considered to be significant. It was then possible to inhibit this interference activity with serum from an asymptomatic W strain artificially infected carrier horse, this carrier serum having been collected around a year after the horse was infected. It should be emphasized that this interfering agent has not yet been proved to be the virus of EIA; work is in

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*Kindly supplied by Dr. Byron Berlin, University of Michigan, Ann Arbor, Michigan.*
progress to confirm that the tenth passage material induces typical EIA in a susceptible horse. This work is continuing.

F. Skin Sensitizations:

The delayed hypersensitivity reaction that occurs with tuberculosis infection has provided a suitable diagnostic method wherein serologic tests have gone wanting. Since EIA is cyclic, suggesting a low level humoral antibody response, it was considered desirable to conduct skin sensitivity reactions. The best type of antigen to use was unknown, therefore, many tissue preparations, whole EDTA bloods, seraums, and centrifuge pellicles from normal and diseased animals were used. Twenty-eight different preparations were heated to 60°C for one hour to inactivate the virus. Ten animals were inoculated intracutaneously with 0.1 ml. of each of the 28 preparations; two of the ten horses were normal uninfected animals. Skin thickness and wheal diameters were measured at intervals thereafter. All values whether being zero or greater were averaged and are presented in Table I. Using analysis of variance of unequal numbers, a computed P value of greater than .0001 between normals and infected was obtained at three days.

### TABLE I
Mean Wheal Diameters of Skin Tests

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two Normals</td>
<td>6.08 ± .95 mm.</td>
<td>1.35 ± 0.042 mm.</td>
</tr>
<tr>
<td>Eight Diseased</td>
<td>10.16 ± 1.59 mm.</td>
<td>4.11 ± 1.16 mm.</td>
</tr>
</tbody>
</table>

A second trial one week later using the same horses plus three additional normal animals resulted in essentially the same differences; in this experiment 18 samples were tested, see Table II. Analysis of variance gives a P value of greater than .0001 at the 48 hour reading.

### TABLE II
Mean Wheal Diameters of Skin Tests

<table>
<thead>
<tr>
<th></th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five Normals</td>
<td>2.50 mm. ± 2.49</td>
<td>.77 mm. ± .87</td>
</tr>
<tr>
<td>Eight Diseased</td>
<td>4.21 mm. ± 1.05</td>
<td>2.44 mm. ± .30</td>
</tr>
</tbody>
</table>

A third trial, Table III, two weeks following the first with only five of what we considered the best preparations were used.

### TABLE III
Mean Wheal Diameters of Skin Tests

<table>
<thead>
<tr>
<th></th>
<th>24 hours</th>
<th>40 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five Normals</td>
<td>1.41 mm.</td>
<td>1.02 mm.</td>
</tr>
<tr>
<td>Three &quot;In incubation&quot;</td>
<td>1.70 mm.</td>
<td>1.50 mm.</td>
</tr>
<tr>
<td>Eight Diseased</td>
<td>2.08 mm.</td>
<td>1.61 mm.</td>
</tr>
</tbody>
</table>
A fourth trial was started four weeks following the first using five preparations; at three days, averages were 1.6 mm. for five "normals," 1.8 for three "in incubation," and 2.7 for two diseased. One day after the fourth tests were begun, it was realized that all "normal" horses that had not been followed very closely had become accidentally infected, presumably from live virus in the skin test preparations. Subsequently four of five of these animals died and it is strongly believed that the old recommended procedure of 60°C for one hour did not kill all the virus; heat inactivation of viruses follows an exponential curve with time and in this situation high levels of virus were being used and presumably not all of the virus was inactivated.

The delayed skin test reactions do appear promising, however, in that in trials one and two there was greater than a three fold difference in wheal size between the normal and the diseased animals in the two or three day reading. The changes in the third trial may have been caused (1) from the infection, (2) from desensitization of the infected horses, or (3) to increased sensitization in the "normal" animals. The size of the reactions suggest that desensitization occurred.

G. Lack of Immunogenicity of Infected Serums:

Immune electrophoresis is a method to detect the presence of the many antigens which occur in serum. It was considered as a method which would demonstrate the presence of EIA virus, virus-antibody complex, or abnormal serum protein in the infected horse. Consequently, rabbits and chickens were inoculated with normal or infected serums, the serums being mixed equal parts with complete Freund's adjuvant,* injected intramuscularly or subcutaneously in one injection. Animals were then bled one month later, and both double diffusion systems and immuno-electrophoresis systems** were set up, using pre- and post-inoculum serums with respective and with crossing antiseraums thereto. In neither system were any lines present in the EIA serums that were not present in the normals; on the other hand, there were more lines between normal horse serum - rabbit antiserum than between EIA serum and its respective antiserum. The reactions between normal horse serum - rabbit antiserum and EIA serum were essentially as good as between normal horse serum - rabbit antiserum and normal horse serum. The deficiency was present in the antiserum against the EIA serum; the reason for such deficiency was not determined - it may be postulated as being due to several causes such as (a) the increased serum lipids present in the sick horse causes the remaining proteins to be less antigenic; (b) the loss in serum albumin in the course of the disease provides for less antigenicity. This appears to be a promising finding that should be investigated further.

V. General Discussion of Equine Infectious Anemia:

Most of the experimental infections in this study were with the

*Difco Laboratories, Detroit, Mich.

**LKB Immunelectrophoresis Equipment, LKB-Produkter AB, Stockholm, Sweden.
Wyoming strain of virus. A few remarks about certain observations in our study are pertinent:

(1) The anemia was not marked in our experimental animals and often in acute deaths not a finding at all. The consistent finding of the altered serum lipoproteins, however, suggests changing the name from equine infectious anemia to equine infectious lipemia. Field infections, however, in Florida were accompanied by packed cell volumes as low as eight percent. The F strain appears to induce more of an anemia than the W strain. To illustrate further, of 12 animals infected with Wyoming strain of virus, seven had no fall in PCV following inoculation and, in fact, had had a rise in PCV's. Five of these seven died within 11 - 28 days after inoculation (average death time of 19.6 days). Five of the original 12 inoculated with W strain virus had low PCV's of 19, 20, 21, 27, and 29 respectively; the three with the lowest PCV's died 18 - 28 days after inoculation (average death time of 23 days). With Florida strain infections, low PCV's appeared somewhat more consistently; all five animals inoculated with F strain had PCV's of 11 - 25 from 39 to 152 days p.i. (average time of lowest PCV was 77 days). Three of these animals died at 39, 55, and 86 days p.i., average death time of 60 days. Four surviving W strain animals have been observed for as long as 3-1/2 months to 3-1/2 years (one - 3-1/2 mo., two - 11 months, one - 3-1/2 yrs.); the three surviving F strain infections have been observed for eight, six, and six months respectively.

(2) Incubation periods: Serums or plasmas, stored at -60 to -80 C, with one exception, were used for inoculation purposes. Quantity of inoculum varied from one ml. 10^{-4} dilution to 80 ml. citrated plasma. Dose of inoculum appeared to have some effect upon incubation period; (a) Three animals receiving 1 x 10^{-3} or 1 x 10^{-4} ml. intravenously had incubation periods of 10, 12, and 13 days (average of 11.7 days); (b) one animal receiving 45 ml. experienced fever in four days. The five F strain incubation periods averaged 8.2 days; the 12 W strain incubation periods averaged 8.6 days.

(3) Different Strains: Three properties in the infected horses suggest differences in virus strains; such differences were not completely confirmed by immunologic means, although the skin test results suggested that immunological types do not exist. (1) Terminal serums or plasmas from four horses dying with W strain infections possessed LDH's of 3900, 19,600, 3725, and 6900; terminal plasma from one horse infected with a Texas strain had an LDH of 4125. Maximum LDH values from three W strain infected horses that did not die were greater than 2000, 1920 and 1400, mean of 1773. Terminal serum from one F strain donkey had an LDH of 1430; LDH maximums from three F strain infections which did not die were 1700, 800, and 1690, mean of 1397. The suggestion here is that the W strain is accompanied terminally by LDH values much higher than F strain infections and in non-terminal cases with values higher than F strain infections. The Texas strain simulates the W strain. (2) Anemia consistently developed in the five F strain infections
as mentioned previously, whereas only five of 12 W strain infections developed anemia of slight degree; in seven of the 12 W strain infections essentially no anemia was present. (3) As seen in the preceding section, three F strain infections died in 60 days; three W strain infections died in 23 days. The W strain induced death earlier than the F strain. The three differences suggest different manifestations of disease possibly de-nearing at least two immunologic strains; the numbers of animals used were too few to be able to make definitive conclusions. 

As mentioned in the paragraph on skin tests, five normal animals were accidently infected in this skin testing procedure; from the nature of the high terminal LDH values and the lack of anemia, we might conclude that the W strain was not heat inactivated and caused disease in the normal horses. The possibility exists of course that deaths here were due to mixed infections. Using the same skin test materials in the horses previously infected with known strains—five with W strain and three with F strain, none of these animals developed fevers within two to six weeks after skin testing manipulations were begun, indicating disease un-responsiveness and supporting the contention that an animal in the carrier state from F infection is "immune" to infection with the W strain. The time of observation may have been too short to be significant; but if one looks back at the original incubation periods, he sees that both F and W incubation periods averaged just over eight days. That W strain was "re-sistant—or immunologically unresponsive" to F strain could not be evaluated in the accidental happenings following the skin tests if we assume that the normal horses became infected with the W strain and not the F strain. Suffice it to say that there is a suggestion in these findings of strain differences but not type differences; clarification on these points must await further investigations.

VI. Summary:

1. Serums from horses infected with the Wyoming strain or a Florida strain of the virus of equine infectious anemia do not contain antibodies to Chang's "lipovirus."

2. Erythrocytes collected during the acute febrile stages of infection to both Florida or Wyoming strain infections are agglutinated by commercially available anti-equine globulin antiserum; such reactions are considered to be positive direct Coomb's tests.

3. Hemolysins were isolated by differential centrifugation from the livers of horses dying with either the Florida or Wyoming strain infection; these hemolysins were not specific for equine infectious anemia since a similar hemolysin was isolated from a horse dying with Babesia caballi infection.

4. Terminal serums from horses with Florida or Wyoming strain infection interfered by as high as $10^{27}$ logs against the encephalomyocarditis virus in mice. An agent, presumably the virus of equine infectious anemia, when passaged ten times in equine thymus cell tissue cultures, interfered significantly with vaccinia virus replication. Carrier serum
RESEARCH FINDINGS IN EIA

5. Infected horses when tested intradermally with various antigenic preparations developed wheal reactions greater than three-fold larger than normal horses; statistical evaluation using analysis of variance resulted in P values of greater than .0001.

6. Serums from the acute stages of infected horses, as measured by immuno-electrophoresis or by double-diffusion precipitin test methods in agar gel, were less immunogenic for rabbits or chickens than were pre-inoculum serums or serums from the asymptomatic carrier stage of the disease.

7. Acridine orange staining of terminal blood examined by fluorescence microscopy revealed the presence of small particles attached to the surface of the erythrocytes; by electron microscopy these particles measured about 150 millimicrons and are called "myelin sheath bodies." Electron microscopy of kidneys revealed the presence of "microbodies"; electron microscopy of the infected livers revealed mosaics with virus-like particles 20 millimicrons in diameter.

8. Histopathologic examination of livers from the asymptomatic carrier may or may not indicate lesions coincident with infection.

9. Serum lipoproteins in the febrile and terminal stages of the disease may (a) become ten fold greater gravimetrically or (b) be ten fold different electrophoretically from normal values.

10. Normal serum lactic dehydrogenase values in horses average around 570; in the febrile and terminal stages values range from 1800 to 19,600.

11. Comparisons of erythrocyte packed cell volumes, serum lactic dehydrogenase values, and death times between Florida and Wyoming strains suggested possible strain differences. Limited immunologic evidence, however, suggested that these two strains are not immunologically distinct virus types.

ACKNOWLEDGEMENTS

The authors thank the following individuals who assisted in this study: Dr. A. J. Kniazeff, Univ. of California; Dr. D. H. Moore, Rockefeller Institute, New York, N.Y.; Dr. C. F. Simpson, Univ. of Florida, Gainesville, Fla.; Dr. J. W. Needham, Chemical Abstracts, Columbus, Ohio; Dr. R. S. Chang, Harvard Univ., Cambridge, Mass., and Mr. Karl Schilling, Kissimmee, Fla., deceased. Grateful thanks are extended to technician, Mr. G. W. Stroup, to secretary, Mrs. M. Draggoo, and to Mr. D. F. Tallman, assistant toxicologist, for their untiring assistance.
Electron micrograph of a crystalline array present in the cytoplasm of an hepatic cord cell from a pony dying with Wyoming strain EIA. Fixed in OsO₄, imbedded in EPON (Luft 8:2); thin sectioned; stained with lead citrate; X 12,000. Particles comprising the mosaic measure about 25 mu.
Electron micrograph of an hepatic cord cell nucleus from a pony dying with Wyoming strain EIA. Fixed in OsO₄, imbedded in SPON (Luft 8:2) thin sectioned, stained with lead citrate; X 21,000. Note the innumerable electron dense particles, 25 mu, throughout the nucleus and inside the encapsulated area.
Electron micrograph of parts of two adjacent erythrocytes from a pony infected with Wyoming strain EIA. Blood was collected from the jugular vein and fixed in OsO₄; imbedded in EPON (Luft 8:2); thin sectioned; stained with lead citrate; X 320,000. Note the body between the erythrocytes suggestive of a "myelin sheath figure"; total dimensions of these figures measure 65-150 mu; periodicity of the figure measures 62.5 Angstroms.

REFERENCES


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES


In evaluating the problems confronting the horse population in the United States the two diseases, Piroplasmosis and Infectious Anemia, quite obviously are the most significant, both because of the magnitude of the current losses and because of the economic threat imposed on the entire industry.

Experience has amply indicated that diseases with these characteristics can be controlled only on the national level and that all efforts should be oriented in the direction of developing effective tools for a national eradication program.

The following equine diseases are presently the greatest handicap to the equine industry and the most in need of research support in the order listed:

1. Piroplasmosis
2. Infectious Anemia
3. African Horsesickness
4. Respiratory Diseases

Development of a standard health certificate pertaining to interstate movement of horses is also an indicated need.

EQUINE PIROPLASMOSIS

Equine Piroplasmosis (EP) has been reported from several countries in Africa, Asia and Europe, and it is reported in latitudes equivalent to our Canadian border.

EP was first diagnosed in the United States in Florida in 1961. Since that time it has been diagnosed in Georgia (four cases on one premise) and in Puerto Rico. The Georgia herd was subsequently eradicated. Diagnosis has been based on finding the piroplasma bodies in blood smears of infected horses or recipient horses inoculated with suspect blood.

The tropical horse tick (*Dermacentor nitens*) is the only proven vector of EP in the United States. Several other tick species have been incriminated as vectors in other parts of the world, therefore, all tick species which infest United States horses should be checked as possible vectors.
Recommendations of this Committee

1. That support be given to the eradication of the disease in Florida with the tools presently available.
2. That support be provided to conduct a survey for EP in areas outside of Florida where there are proven vectors.
3. The Complement-Fixation test and the agar-gel precipitin test now in use appear useful and practical but need continued refinement in application and interpretation. The development of improved or new diagnostic tests should be encouraged. It is now apparent that Ristic's new passive hemagglutination test is well worthy of field evaluation.
4. That support be given evaluation of therapeutic agents for the prevention, control and treatment of EP caused by either Babesia caballi or Nuttallia equi.
5. That research be conducted to identify EP vectors in the United States.
6. That the current vector survey (cooperative State-Federal) of equidae in the United States be continued.

EQUINE INFECTIOUS ANEMIA

Equine infectious anemia continues to be one of, if not the most, important disease of horses for which insufficient information on diagnosis and control is available.

The threat of this disease is important, not only from the standpoint of its effect on equines, but as a threat to a sizeable source of tax revenue in those states participating in horse racing. Since 1900 EIA has been reported throughout the United States and during the last five years in all but five states where it was possibly unrecognized. EIA is also present in 28 foreign countries. The disease occurred in epidemic proportions in 1947 at Rockingham Racecourse, resulting in 77 cases in approximately 900 horses, with serious effects on racing in the Northeast that year. In 1965 the disease appeared in racing thoroughbreds and other horses in several states. At one track there were 38 suspected cases, 25 confirmed, with 20 deaths. The mobile nature of horse activities provides annual opportunity for the exposure of a large percentage of the United States horse population to this disease.

In areas where the disease is endemic, continual losses are to be expected.

Research needs for this disease include, at the top of the list, a more critical laboratory diagnostic test. The precipitin test, employing the serum of a suspected animal reacting with serum from a rabbit or sheep that has been inoculated with antigenic material, has given promise. This test is reliable in the detection of active cases and a high percentage of quiescent carriers. Until a test is developed which will detect all carriers at any phase of infection we lack the tool required for conducting an eradication program. To date, the results of a critical evaluation of this test have not been published. This test was introduced to the United States
by Saurino, and has been further developed by Moore and Livingston. A report on studies of serum proteins, lipoproteins, histopathology of the liver, viral interference tests, dermal sensitivity reactions, and hematological examinations is reported by Gainer at this meeting. All of these examinations have some value, but none give positive results each time the test is applied in infectious anemia.

Fundamental information regarding the nature of the infectious anemia virus is essential. The usual techniques employed for the concentration of viruses have not been successful with the infectious anemia agent. The development of a vaccine against the disease is essential. The ease of spread of this disease by contaminated syringes and needles necessitates the use of disposable equipment of this nature.

Current research activity is limited. The investigations of Gainer and Amster of the Florida Department of Agriculture aided by the National Institutes of Health, and of Moore and Livingston at Texas A & M University are the only reported projects current in the United States. During the last ten years only the Japanese, as a nation, appear to have had a vigorous research effort on this disease.

Control

Regarding the control of this disease, it would seem that a condition that spreads as slowly as equine infectious anemia would offer hope of eradication should a more critical diagnostic test become available. Several states have reported that official quarantine and regulatory activities are instituted upon discovery of a case of infectious anemia. Obvious difficulties will be experienced in eradication of a disease involving animals with the monetary and sentimental value of horses. Funds for indemnity of equines will also pose a problem in some states.

Although EIA is the most devastating and costly disease of horses in the United States support for research has been grossly inadequate.

Successful control and eradication efforts appear to await the successful result of a vigorous research effort to develop a critical diagnostic test. This Committee recommends that (1) the United States Department of Agriculture provide support for the study of EIA; and (2) other foundations and institutions be urged to activate coordinated studies of this disease.

RESPIRATORY DISEASES

The Committee recognizes the importance of respiratory diseases and commends the equine industry for contributing to research for their control.

IMPORTATION

There is a need for increased vigilance at ports-of-entry, with particular emphasis on ticks entering on zebras and other imported solipeds. Additional testing should be conducted at quarantine stations, and if test
tools are not adequate or available, research should be stimulated to develop more adequate diagnostic procedures. Special emphasis should be placed on detecting possible carriers of equine piroplasmosis, or horses infected with African Horsesickness at all quarantine stations.

REFERENCES

Now getting down to this subject of the conditions for the importation of cattle from approved countries in Europe into Canada, needless to say the problem has been under study for a long time, and as you are all aware, each of us in our respective countries have been under pressure to do something about this over the years, and we have stoutly over the years felt that it could not be done. We have never said that it would not be done; we have merely said that it could not be done because of the safety factor of bringing foot-and-mouth disease into this country. At the same time as we have said this, we have also assured our people, our livestock industries, that the problems would be kept under constant study and if and when this became possible, then something would be done about it.

Now I think the first point of interest is that there is a basic difference between the Canadian and United States law with respect to importation of the cattle from other countries. However, we have never specifically prohibited the importation of animals from any country such as yours. The United States law has certain specific prohibitions—it is not my place to of course discuss this law but I must point out this basic difference; so that for this importation into Canada of cattle from countries not normally considered as traditionally free of foot and mouth disease, there was no necessity for changing the basic requirements or regulations of our law, because the provision for such importation has always been there, providing we could satisfy ourselves that adequate conditions could be met for such importations without exposing Canada or the North American continent to disease. So, that as a result of a careful study, which included not only our own authorities but world authorities with respect to foot and mouth disease such as Dr. Brooksby of the Perbright Research Institute in Britain; Doctor Callis and his people of the Plum Island Research Institute. It was finally concluded with the improved techniques and the evaluation of these techniques together with exposure and quarantine, that if a set of conditions could be laid down and if these conditions were to be followed implicitly, then the importation of animals could be made without undue risk. Now this also included of course, the country of origin, and I think one of the things which has not been clearly understood—that the proposed importation of cattle into Canada is not a carte blanche operation whereby cattle can be moved from any foot-and-mouth infected country into Canada. The first criteria is that the country must

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be selected and approved in order that we can satisfy ourselves that foot and mouth disease has been brought under some reasonable level of control in the country with which we are dealing, and as a result of this study and as a result of the quest for permits for the importation of cattle from France, the French situation which in fact all countries just the same as your own people are continually reviewing the foot and mouth situation in all countries—as a result of these reviews, it was finally agreed from our point of view that France has reached the point where we could consider these importations. As an example, France now has a slaughter policy for foot and mouth disease. It is true that they still vaccinate; they require the vaccination of all calves against foot and mouth disease at six months of age, but they carry on with their slaughter policy and have had this slaughter policy over the past four or five years. In 1963 there were 28 reported cases of foot and mouth disease in France. In 1964 there were a total of 46. In 1965, for the first half of the year, when we began our importations there were only seven reported cases of foot and mouth disease in France. So that, this is the first point I want to make: in this importation it is not a matter of simply saying that they can come from all countries. When we receive a request, as we did in the case of France, or those owners wishing to import from France, the first operation is for us to examine carefully the country and see whether in our opinion it is possible for us to get cattle from that country, which have not been exposed to or which have not in any way had the foot and mouth disease. So that I think this is the first clear point that I would like to make—that this does not merely say that cattle can be brought in from all or any countries.

Now, we are of course very cognizant of our responsibility with respect to disease control; we recognize that we cannot operate as an isolated unit in Canada; we must cooperate in disease-control matters on the North American level rather than on an individual country level, because what we do is of concern to you people; and how the United States feel, of course is of serious concern to us. The North American continent, is by and large with the exception of Australia and New Zealand the really only large major continent in the world left which is not exposed to these conditions—so that we are, I want to assure you, sincerely cognizant of the dangers and problems involved with respect to any exposure to foot and mouth disease and those considerations have been very, very carefully studied when our decisions were made to make the importation. In addition to the problems of, or rather the advances in geological techniques, the advances of improved control in France in this particular case, we were faced with another problem and that is that cattle were moved from France to French islands of Saint Pierre and Miquelon which are within ten miles of the shores of our eastern province of Newfoundland. Now, these cattle were brought there, and it is true and we have to admit that a sincere effort was made to test these cattle for these various diseases, including foot and mouth disease, but these tests were not done with any surveillance on our part of the part of the United States officials, and therefore we in Canada were faced with the problem that cattle could and
had been brought from the continent of Europe to within ten miles of our shores, over which we had absolutely no control whatsoever. On the islands of St. Pierre and Miquelon, there is no feed whatsoever for cattle; there is no manner to dispose of them on the island other than to eat them, and the quality of the cattle that were brought to St. Pierre and Miquelon certainly weren't brought for eating purposes because it would run into very expensive beef. So that, when we had to face the problem that cattle were being brought under conditions over which we had no control, to within ten miles of our shores, now this didn't in fact decide the issue that something had to be done but it became apparent to us that one could no longer keep our head in the sand and simply reject the advances in serological techniques and the advantages in disease control without doing something about it to make importations legal. If they were not going to be done legal, the pressure was mounting and the opportunities were available where one might consider that importations might be made illegally.

Now let me say first of all that we are not concerned with breeds. There has been much talk about the so-called breed that's being brought into Canada at this time. The Health of Animals Branch of Canada's Government are not in any way concerned with what breeds of cattle are brought in. If in fact, our livestock industry want to cross their cattle with Himalayan goats they can do it, as far as I am concerned, providing the goats are healthy; so that we do not enter into the discussion of whether in fact this breed is necessary for the improvement of our beef-producing situation in Canada and the North American continent—we are concerned solely with disease matters, and once having satisfied ourselves that these disease matters could be overcome, then it was incumbent upon us as veterinarians to simply lay down conditions which would do it, and if they could meet these conditions then let it be done. If in fact, gentlemen, this were not so they wouldn't need veterinary offices at either the Federal or the State level—if we are simply going to sit back and shut our eyes to advances which make freer international movement of livestock available, then Governments should simply hire clerks and train them to say "no," and you would get them an awful lot cheaper than they would veterinarians, although I must admit they don't pay veterinarians too much either. But nevertheless I think that we have to recognize as a professional body that as advances come along then it is incumbent upon us—in fact our bounden duty—to make these advances available for the improvement of the livestock industry. Well, you are all aware of the result of these studies of course, which we made and which we sat with the United States officials and discussed—as a result of these of course, the United States people brought out their semen import regulations with which we are in total and hearty agreement—and should anyone wish to apply those semen regulations and bring semen into Canada if in fact they can meet the requirements as laid down by your people, then we are in hearty agreement with them.

While the United States authorities were considering the problem of semen, we at the same time were going along attempting to consider the
METHOD OF IMPORTATION OF ANIMALS

problems of cattle, and as a result of these considerations we arrived at the set of conditions under which cattle can be brought into Canada. Now I will go directly into the conditions and because of time I shall try and move through them very quickly. The conditions of course apply to all diseases; I think that we can readily take for granted that the normal tests for Brucellosis, Tuberculosis, and things of that nature are pretty well accepted on an international basis and in all probability provide the protection we need from that point of view—so that our primary concern is of course with the foot and mouth disease. Now, the conditions first of all specify that cattle should be moved from the country of origin to an official quarantine station in that country. The quarantine station in the country of origin must be approved for the purposes by the Veterinary Director General of the Canada Department of Agriculture. Cattle intended for importation into Canada shall be inspected in the country of origin by a Veterinary Officer of the Government of Canada on the farms of origin and during the quarantine period so that we have had a Veterinary Officer in France since the middle of last June. Before entry to the approved quarantine station, a certificate issued by a duly authorized Veterinary Officer of the country of origin are to be furnished, to the effect that he has inspected the animals and has found them free from any evidence of infectious or contagious disease and that no Foot and Mouth Disease or other serious infectious or contagious disease affecting cattle existed on the premises of origin, or within ten miles of the premises of origin for a period of nine months immediately prior to the quarantine in the country of origin; that no animal on the premises which was susceptible to the virus of Foot and Mouth Disease was exposed to the disease during the nine months prior to the date of inspection of the animal; that the country of origin is free of Rinderpest, contagious pleuropneumonia, and such other diseases as may be specified. In other words, we would not consider taking cattle from countries which have Rinderpest, contagious pleuropneumonia, and some of the other serious Epizootic which is not totally possible to test for under today's technological circumstances. That the cattle had passed a negative test for tuberculosis, and brucellosis, and that insofar as can be determined vibrio fetus and trichomoniasis infections are not present; that the cattle at the farm of origin show no critical evidence nor the presence nor history of leucosis and the haemological examination with respect to leucosis must take place—all of this on the farm of origin prior to even entering the quarantine station. That leptospirosis has not been diagnosed in the cattle or herd of origin within the previous 12 months and that the cattle themselves being selected for export have been subjected to the nine common serological tests for leptospirosis with negative result; that subsequent to the commencement of the tests prescribed, the animals were kept separate from all other animals while on the farm of origin; that the cattle have at no time been affected with or exposed to foot and mouth disease; had not been vaccinated against it and that serological tests were carried out with negative results within thirty days before leaving the premises of origin; that the cattle originated on the premises on which Johne's Disease
is not known to exist—well, then it goes into the other diseases and insofar as can be determined Blue Tongue has not existed on the premises of origin.

Now, inasmuch as France requires the vaccination of cattle at six months of age, it was necessary for us to say that no cattle could be moved into the Brest quarantine station which took place on September 1st, and which were over nine months of age. In other words, the French are prepared to eliminate the necessity of vaccination of calves at six months of age, which are brought for export purposes—other than that, they must be vaccinated; therefore, those calves selected for export purposes can be withheld from the vaccination requirements of the country, but if we were to go beyond nine months of age, then it would be impossible for us to assure ourselves that the cattle had not been exposed to infection—in other words, when one seeks a history from a livestock owner, if you go very much beyond nine months or a year, you are dealing pretty much in the realm of fiction. So we've required that the calves be born during the calendar year of 1965, which means that no calf could have been more than nine months of age, and this means that we could have some control over the fact that they were not vaccinated and of course the serological test would pick up the antibodies of vaccination under any circumstances.

Now, the conditions of quarantine in the country of origin, the quarantine station of the country of origin, must have been thoroughly cleansed and disinfected in a manner satisfactory to our own veterinary authorities. Transport of the cattle from the premises of origin to the quarantine station in the country of origin must be effective and specially disinfected, mainly with trucks or mechanically propelled road vehicles by the most direct available route, and in fact they were taken under convoy with our Veterinary Officer being with them. All cattle intended for importation should undergo a quarantine in the approved official quarantine station for at least thirty days—that is, thirty days in the Brest quarantine station in France, under the joint supervision of a Veterinary Officer of the country of origin and a Veterinary Officer of the Government of Canada—in other words, the control of the quarantine station in the country of export was under the joint supervision of a Canadian Veterinary Officer and a Veterinary Officer of the French Government. Calves in the quarantine station shall be subjected to serological and other tests as prescribed for foot and mouth diseases (I'll go over these tests a wee bit later) under the Canadian Veterinary authorities and found negative before shipment to the Canadian Quarantine Station is authorized. Temperatures of all cattle in the Quarantine Station shall be taken twice daily; all feed and litter for use in the Quarantine Station shall be supplied directly from Canada or from a country considered free from Foot and Mouth Disease and other serious epizootics. Now, in actual fact, we shipped all of the feed, hay and straw for the cattle during the Brest Quarantine Station from Canada, we shipped it from Canada to France; it was met in France by our Veterinary Officer and convoyed from there to the special clean and disinfected quarantine station, so that all feed, hay and bedding which the cattle used
during the quarantine station sojourn came from our own country.

On completion of the quarantine period, the cattle shall not be accepted for shipment to Canada unless the Veterinary Officer of the country of origin and a Canadian Veterinary Officer jointly issue a certificate to the effect that the cattle have been kept under quarantine, under their supervision, for the stated period, and that during that time they remained healthy and showed no clinical signs of any contagious, infectious or parasitic disease affecting cattle, and that all applicable requirements have been met.

I am pleased to tell you that the quarantine was very, very closely kept. I was at a meeting in Kansas City not too long ago and some United States people decided that they would spend some of their surplus funds and go over to France and look at these cattle in the quarantine station. Afterward they came to me quite indignant and said after having spent the money to fly over to France from the United States they were summarily turned away from the door and told that they couldn't come in by our Canadian Veterinary Officer. I was quite pleased, in fact that they had been turned away and really didn't shed a tear for the cost of their air travel. The cattle shall be moved under Veterinary Supervision direct from a quarantine station to the vessel for export in specially disinfected mechanically propelled road vehicles—in other words, moving them from the quarantine station to the ship in France, was under our control. The cattle must have been moved to Canada by sea (we do not permit importation by aircraft) and of course the vessel has to be clean and disinfected; again the hay, straw, the feed, everything that the cattle are concerned with and use during the ocean voyage was supplied from Canada and shipped over to France for the purpose of feeding the cattle on the way back. At the Grosse Ile quarantine Station, the landing shall be carried out in accordance with instructions as the Veterinary Director General may give—in this case, we could not of course permit the cattle to come into a normal port. Inasmuch as there is not adequate landing facilities or docking facilities on Grosse Ile the cattle had to be unloaded in midstream onto barges, and taken by barges from the ocean-going vessel that was a mile out from shore onto the island. Grosse Ile is an island about thirty miles south of the City of Quebec in the St. Lawrence river; the St. Lawrence river at this point is about 15 miles wide so that there is roughly seven miles of water on each side of the island. The vessel from which the imported cattle have landed shall be immediately cleaned and disinfected; the calves shall be kept in the quarantine station for a period of at least 90 days after arrival under the supervision of a Veterinary Officer; this is in accordance with our law which requires a minimum of 90 days; in actual fact, by the time we get all of the necessary tests done, the quarantine period will run about six months. This is aided and abetted, of course, by the simple fact that in the winter time it is impossible to get a ship other than an ice breaker into or out of Grosse Ile so that there is no way of getting the cattle out until the spring break-up, so that the quarantine period will in fact be the minimum required by the law of 90 days—the actual quarantine period will run closer to six months. During
the quarantine period the cattle shall be subjected to serological and other tests, Probang and Mouse inoculation for Foot and Mouth Disease, blood agglutination for brucellosis, the whole rigamarol—would be all done again insofar as the brucellosis, leptospirosis, leucosis, tuberculosis,—the ordinary series of tests to which we are all familiar with, and of course the test for Blue Tongue will be carried out. The test for Blue Tongue is a bet of a difficult one where we have to take blood from the cattle and inoculate the serum into sheep over a three month's period (three inoculations) and then take blood from the test sheep, separate the serum, and send serum to Ondersterport, South Africa, and the Blue Tongue Laboratory there where they will run serological tests on it for Blue Tongue.

Now, the next three sections I think are important and worth reading in detail. If any or all of the imported cattle or test animals are not classed as negative to any of the tests, the Canada Department of Agriculture may cause any or all of them to be slaughtered, and the carcasses disposed of as considered fit without compensation to the owner. If any or all of the imported or test animals show clinical symptoms of the disease the Canada Department of Agriculture may cause any or all of them to be slaughtered, and the carcass or carcasses disposed of as considered fit without compensation to the owner. Now, if any or all—if any or all—of the imported or test animals show clinical symptoms of Foot and Mouth Disease, the Canada Department of Agriculture shall cause all of them to be slaughtered and the carcasses disposed of as considered fit without compensation to the owner. Well, the disposal of the carcasses, of course, should such an event occur, but should it occur, of course the disposal of the carcasses will be by incineration on Grosse Ile. And no cattle shall be moved from the quarantine station until duly discharged thereunder from the authority of the Veterinary Director General of the Health of Animals Division of the Department of Agriculture of Canada.

Following the discharge of the imported cattle from the Canadian Quarantine Station, on Grosse Ile imported cattle and any or all offspring shall be added to a Canadian herd of susceptible cattle approved for this purpose by the Veterinary Director General. This Canadian herd, together with the imported cattle, shall be placed under Departmental Quarantine for a three month's period following release from the Grosse Ile quarantine. The imported cattle shall be free to move without restriction.

Those are the detailed conditions which have been laid down.

THE TESTS TO BE USED

There are four serological tests which are being used for this operation: The Agar Gel Precipitation Test was done by the French authorities
on the cattle on the farm of origin. The Cell Metabolic Inhibition test is the second test. The Virus Neutralization Test in suckling mice is the third. And the Probang Test is the fourth.

Now, then the Agar Gel Precipitation Test took place on the farms of origin by the French authorities. At the same time, our Veterinary Officer took blood and sent serum to the Perbright Research Foot and Mouth Research Institute in England, where the Cell Metabolic Inhibition Test was conducted on the cattle. All cattle showing any evidence of reaction whatsoever regardless of whether the reaction was considered to be at an infection level or not—in other words even as false positives of which they come in this test were discarded so that there was no tolerance given to the tests whatsoever. In the Brest quarantine station, again the Cell Metabolic Inhibition test and the Virus Neutralization Test in suckling mice was carried out at the Perbright Research Foot and Mouth Research station in England, and again no tolerance whatsoever was allowed insofar as the tests are concerned, in spite of the fact that we recognize that false positives at very, very low titer levels readily come up in these kind of tests, and in fact if we tested Canadian cattle and United States cattle by these tests we would get these low level false positives. But for our purpose, we simply said that any reaction whatsoever must be discarded in spite of the fact that it did not in any way indicate infection. At Grosse Ile again, the Cell Metabolic Inhibition Test and the Virus Neutralization Test in suckling mice would take place with serum being sent to Perbright for the purpose of those tests, and we will do the Probang Test on mice at Grosse Ile. Now the Probang test as some of you are aware is rather a recent development from Holland and recently we find whereby material is taken from the tonsils of the suspected, or the cattle under test, and this material is injected into unweened mice, so that those are the tests that will be used and the places at which they will be used.

Now this means that each animal being imported will have had seven negative serological tests for foot and mouth disease; in addition to the seven negative serological tests and the quarantine period of six months in Grosse Ile and a month in Brest and twelve days on the ocean, there will be 30 test animals of Canadian origin put into the quarantine station in Grosse Ile and they will live in amongst and with the imported cattle for a period of six months as susceptible test animals. Now, in order to carry all of this out it means that we have had to carry out approximately twelve hundred individual serological tests and by the time we finished we will have used somewhere in the vicinity of 20,000 unweened or suckling mice in order to carry out the inoculations. Now there were 65 permits issued originally for the importation of 121 cattle. There were originally selected in France by Canadian importers 254 head of cattle. As a result of reactions to the various tests and rejections by our Veterinary Officer on farms wherein he wasn't satisfied that he could get a history which satisfied him with respect to the cattle, there were 144 of the original 254 rejected which left 110 head of cattle to come to Canada. Now, the time table on this is that the owner started to buy the cattle last June in France; our Veterinary Officer went over in June; he stayed right with the
owners and visited all of the farms. The cattle were bought and tested in July and August; they moved into the Brest quarantine station and the first about the seventh and eighth of September last; they left the Brest quarantine station—110 of them—again under our Veterinary Officer on the ninth of September, and they arrived at Grosse Ile last Thursday and were taken off the ship about two o'clock in the morning and taken to the Grosse Ile quarantine station where they are now—110 of these Charolais cattle commencing their six-months quarantine period.

As I have said earlier, the whole circumstances, the conditions were discussed with the United States authorities and were very grateful to them for their consideration of them and their suggestions and improvements, refinements which in fact they suggested, and we were very pleased to accept. We invited the United States authorities because we recognize the genuine interest of the United States authorities in this matter. We invited them to send an observer along with this operation and we are grateful that the United States authorities accepted the invitation. We had the United States Veterinary Officer with our Officer in France taking part in observing the operations and we now have the United States Veterinary Officer at Grosse Ile who will stay there along with our own Veterinary Officer for the winter months. I am not so sure what they'll do all winter in Grosse Ile when the snow and ice comes in; television is not too good; there's no one living there insofar as general company is concerned, but they will have the company of a lot of cattle, and I suspect that they'll be with them quite often.

This then is the program, these cattle will leave Grosse Ile at the end of April or the first of May next year; they will then go to Canadian farms for May, June and July—90 days, which means that the end of July in 1966 the cattle will be free insofar as disease or control restrictions are concerned by the Canadian Government. This will mean that the cattle will have been under our close scrutiny and direct observation for a period of 13 months from the time they were first selected by the Canadian importers until they are free to give to them.

In closing, gentlemen, I again want to assure you that we of the Canadian Department of Agriculture, the Health of Animals Branch, are vitally concerned, in fact have just as much concern with respect to the possible introduction of Foot and Mouth Disease as you people do in the United States. We are highly cognizant of our responsibility not only for the Canadian health status of our livestock, but also of the health status of the livestock of the North American continent; we intend to play our full part in maintaining this health status, and I can assure you that everything will be done to be absolutely certain that no infection comes into this country. We have, not only into this country but our stake in this thing is of course considerably greater than yours because if anything did happen we would be the first to have it happen in our country and we don't intend this to happen.

So that, in closing, I just want to give you our full assurances that every precaution will be taken and that every assurance will be given so that we trust you will rest easy. Thank you.
Voice: Doctor Wells, just what is this Probang Test?

Doctor Wells: Now, the Probang Test is merely a matter of taking a Probang and shoving it down the animal's throat and picking off a wee piece of material from the tonsil, and this material then has to be injected, immediately within an hour or two hours at the most, because it spoils rather quickly—into suckling mice. Ordinarily, the mice should live; if any number of the mice die, then obviously they have to be frozen and shipped to Perbright and to establish the fact that foot and mouth virus was not the cause of the death of the mice. You might wonder why we are having the tests done at Perbright, England. As you know, Sero- logical Neutralization Tests require the use of live virus and we in Canada do not have foot and mouth live virus even at our research laboratories—; therefore, rather than import the virus and use it in the country for serological purposes, we felt that it was far better to arrange to have the tests done by the Perbright Foot and Mouth Research Center, which in fact is the world's Titan Center for foot and mouth viruses and considered to be one of the world centers (they've been at the business for about 40 years), so this is why the tests are being done there rather than at home. We simply don't want to import the virus into Canada in order to carry out the serological tests.

A Voice: Doctor Wells, what is the cost per year to ship these cattle into Canada?

Dr. K. F. Wells: Well, it can only be estimated; let me say in the first place, there is no charge for the use of the quarantine station by Canadian importers. The laws governing the importation of livestock into Canada are considered by the Government to be mandatory laws for the good of the nation, and therefore they assume that no individual forced to follow such laws designed to benefit the nation as a whole—should be forced to bear capital costs of a station. Therefore, the only thing that the importer has are in quarantine; now, in addition of course, he has to pay freight and insurance and all that sort of thing. But the total cost to the importer, exclusive of freight and insurance, will run somewhere around $1500.00 a head. Are there other questions?

Question: Are you anticipating starting another importation next Spring?

Doctor Wells: Yes—we anticipate that we would likely have possibly a shipment each year; that is, that they would be as they were this year, selected—moved through the machinery during the late summer and come in the Fall and go through the Winter Quarantine Station again. The Quarantine Station that we have built on Grosse Ile we think is an excellent quarantine station; it is of course totally birdproof and flyproof; it's insulated, electrically heated, automatically controlled for temperature and humidity; the cattle aren't going to suffer too much. Are there further questions?
REPORT OF THE COMMITTEE ON LAWS AND REGULATIONS

G. B. Rea, Salem, Oregon, Chairman; D. E. Flagg, Bismarck, North Dakota; F. W. Hansen, Jr., Hyattsville, Maryland; J. F. Huddelson, Topeka, Kansas, T. A. Ladson, Olney, Maryland; H. J. Rollins, Raleigh, North Carolina; D. L. Smith, Indianapolis, Indiana; W. M. Thompson, Phoenix, Arizona; S. B. Walker, Austin, Texas

Most of us viewed with alarm the original announcement that Canada was opening their Grosse Ile Quarantine Station to European imports. Many of us were chagrined when the Congressional bill was prepared which would give the federal government virtual control over all slaughtering of animals for human food, whether or not these products would enter interstate channels of trade. Some of us were highly incensed when a proposed change in the Uniform Methods and Rules concerning the interstate movement of bison with unknown brucellosis status was proposed.

These subjects precipitated a meeting of the Committee on Laws and Regulations at the Heathman Hotel, Portland, Oregon, July 13, 1965. This meeting was held in conjunction with the National Assembly during the American Veterinary Medical Association Convention. No action was taken by the Committee at that time but the occasion provided a good sounding board for the above subjects which since have been officially considered.

THE GROSSE ILE QUARANTINE STATION

Several letters and one resolution requesting that restrictive action be taken against livestock being imported into the United States from Canada have been received. The subject has been publicly discussed and editorialized in our newspapers and livestock journals. The original alarm at the official Canadian action was considerably modified, when it became known that French cattle could be imported to French owned islands within seven and nine miles from Canadian shores; and this without benefit of any tests or surveillance. Many of us have since applauded the Canadian Government for energetically taking the only action left to them to afford some major protection, not only to themselves but to the United States and Mexico. The United States Department of Agriculture has carefully reviewed and evaluated the Canadian plans and procedures for the importation of cattle from certain countries where foot and mouth disease exists. It was the considered judgment of the Department's scientist that these procedures, if followed in every detail, would not be a means of introduction of foot and mouth disease into Canada and other countries of North America. This Committee also has reviewed these plans and procedures and finds no reason to take or recommend additional restrictive action with respect to Canadian cattle. However, because of the continuing existence of cattle of undetermined health status...
on the French Islands of St. Pierre and Michelon, in close proximity to North American shores, this Committee recommends that the United States Livestock Sanitary Association urge the Canadian authorities to facilitate the earliest possible removal of these animals.

TASK FORCE COMpendium ON MEAT INSPECTION

A bill to expand federal control of all slaughtering facilities, even those under state financed meat inspection, was introduced in Congress October 19, 1965. The Committee has not had an opportunity to review this bill but recognizes that there is a great need for surveillance of slaughtering facilities; ante and post mortem inspection of carcasses for human food, and meat processing inspection in those states which have not seen fit to provide such protection for their consumers. However, this Committee recommends that all states demand equal authority with the federal government in assuring that the high standards presently set by the federal meat inspection service are maintained or at least constantly and consistently sought in those plants which do not enter into interstate commerce. This authority and responsibility should not be solely vested in the federal government and should only be assumed by that agency when local authority fails to function.

PROPOSED CHANGE IN PART 78, Sub Part E, Sub Section 78.20 CONCERNING UNIFORM METHODS AND RULES REGARDING "MOVEMENT OF BISON FOR PURPOSES OTHER THAN SLAUGHTER"

In February 1964 bison were placed under the provision of the federal regulations pertaining to the interstate movement of livestock with relation to brucellosis. Approximately 16 months later a proposal was made to change the aforementioned regulation to permit the qualification of bison at destination rather than point of origin. This is a radical departure from sound disease control methods and procedures. This Committee recommends that the United States Livestock Sanitary Association go on record as being opposed to the proposed amendment and that the Department of Agriculture be so advised.

INTERSTATE LIVESTOCK HEALTH REGULATIONS AND LIVESTOCK MOVEMENT

This is a subject that is discussed not only at our annual meeting but at every regional and area meeting where two or more regulatory officials get together. This is natural and as it should be for it is one of our prime responsibilities. However, since much time and concerted effort has been spent during the past years trying to develop a uniform set of regulations to no avail, it is the thinking of the Committee that the subject should not be officially pursued at this time. This does not mean that we should forget or be unconscious of the need, but rather that a break or pause in the effort may precipitate an entirely new and different approach to the problem, which apparently is the necessary ingredient to a satisfactory
solution. Continued effort toward conformity on an area or regional basis is still recommended.

Your Committee has under study a proposed uniform format for each state's import requirements for livestock and poultry which would be used in printing the ARS 91-17-4 "Health Requirements Governing the Interstate and International Movement of Livestock."

This Committee, a year ago with the assistance of past President Rosner, presented and had approved a Uniform Health Certificate form. This form has been completely adopted by some states and in part by others. Some states have certificates which are similar to the approved form and are waiting the need for new printings before complete adoption is final. We would hope that in the future as needs for changing the form become apparent, suggested changes would be formally presented through the Laws and Regulations Committee of this Association so that official action could be taken and all states be uniformly advised, rather than have arbitrary changes made by some while unknown and unwanted by others.

There is a continued dissatisfaction with the use, as health certificates, of the federal form ADE 2-48 and other forms presently used for release of livestock from federal stockyards. Your Committee has been advised that changes in forms are being considered. It is our recommendation that the ANH be strongly urged to use the recently adopted uniform Health Certificate.

IDENTIFICATION OF LIVESTOCK IN INTERSTATE COMMERCE

This subject broaches a problem equal to or greater than the seeking of uniform health requirements and regulations. This fact should not cause us to avoid the issue. There are those among us who feel strongly that such identification would greatly enhance our regulatory work.

Since no workable suggestions have been forthcoming, the Committee recommends that a joint sub-committee from the Committee on Laws and regulations and Stockyards, Markets and Transportation be appointed to give concentrated study and make recommendations for identification of livestock for disease control traceback purposes for submission and consideration by the Executive Committee of this Association.
PERSISTENCE OF DDT IN MILK AND BODY FAT—A CASE REPORT

A. W. Avens,* H. E. Nadler,** J. G. Matthysse,*** and D. J. Lisk***

In February, 1964 a herd of 40 lactating cows owned by a New York dairyman were quarantined by the New York State Department of Agriculture and Markets because of DDT residues in their milk. It was suspected that one of the probable sources of contamination was sweet corn silage which had been purchased, ensiled, and fed for several months prior to milk sampling. The concentrations of DDT and DDE in the silage sampled in February were 1.98 and 0.13 ppm., respectively. All other sources of feed were analyzed but were found to contain no insecticide.

Milk was sampled periodically during the next ten months and analyses by electron affinity gas chromatography were made to study insecticide disappearance. The milk samples for analysis were a composite of milk from the entire herd. The milk samples taken from January through May (Table I) were analyzed by the Mills extraction and isolation procedure at the Albany laboratory followed by gas chromatography. Those milk samples taken from July through November were analyzed at the Geneva laboratory using electron affinity gas chromatography after the following extraction procedure. One hundred grams of milk was blended with acetone and filtered. The filter was rinsed with acetone until a total volume of 500 ml. of filtrate was collected. Two hundred ml. of the filtrate was partitioned into 100 ml. of hexane and the hexane was injected for analysis. The recoveries of 0.1 ppm. of DDT and DDE added to

<table>
<thead>
<tr>
<th></th>
<th>DDT</th>
<th>DDE</th>
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<tbody>
<tr>
<td>Date</td>
<td>ppm.</td>
<td>ppm.</td>
</tr>
<tr>
<td>1/30/64</td>
<td>0.90</td>
<td>0.18</td>
</tr>
<tr>
<td>1/10/64</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>2/20/64</td>
<td>0.31</td>
<td>0.10</td>
</tr>
<tr>
<td>3/10/64</td>
<td>0.45</td>
<td>0.22</td>
</tr>
<tr>
<td>4/6/64</td>
<td>0.23</td>
<td>0.15</td>
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<tr>
<td>5/18/64</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>7/13/64</td>
<td>0.03</td>
<td>0.07</td>
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<tr>
<td>8/17/64</td>
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<td>0.10</td>
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<tr>
<td>9/22/64</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>11/9/64</td>
<td>&lt;.01</td>
<td>0.08</td>
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TABLE I
Disappearance of DDT and DDE in Composited Milk from Cows Fed Corn Silage Containing DDT

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milk were 100, 100, and 120 and 80 percent respectively. The method was sensitive to about 0.01 ppm. of either DDT or DDE.

Table I lists the residues of DDT and DDE found in composited monthly samples of milk after silage feeding ended (about February 10). The concentration of DDE remained quite constant throughout the sampling period.

Some observations regarding results of analysis of milk from individual cows should be mentioned. The slow rate of DDT and DDE excretion was well demonstrated by the numerous individual cow milk analyses made to enable individual cattle to be released from quarantine. Individual cow sampling began June 3 or about 130 days after the feeding of the contaminated silage had stopped. During this sampling period nine cows ranging from .06 ppm. to .14 ppm. total as DDT in their milk decreased in output to .02 to .05 ppm. in 34 to 41 days. However, one cow increased from .07 ppm. to .08 ppm. in this same time. In 89 days, three cows producing .09, .10 and .14 ppm. decreased to .02, .03 and .08 ppm. respectively, in their milk. In this same time two cows producing .07 to .11 ppm. decreased to .11 and .03 ppm. respectively. In 159 days three cows producing .17, .22 and .30 ppm. decreased to .08, .03 and .04 ppm. total as DDT respectively in their milk.

Table II lists the residues of DDT and DDE found in the fat of various internal organs of two animals which were sacrificed in November, 1964 at the end of the milk sampling period. These animals were chosen based

**TABLE II**

The Results of Laboratory Analysis of Beef Fat Samples for Pesticide Residues are Given

<table>
<thead>
<tr>
<th>Cow #3557 - Dry when slaughtered 11/11/64</th>
<th>Milk 7/64 contained 0.28 ppm. DDT</th>
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<tbody>
<tr>
<td>DDE as DDT ppm.</td>
<td>DDT ppm.</td>
</tr>
<tr>
<td>Kidney 2.30</td>
<td>1.28</td>
</tr>
<tr>
<td>Omentum 2.50</td>
<td>2.10</td>
</tr>
<tr>
<td>Back 1.52</td>
<td>1.14</td>
</tr>
<tr>
<td>Round 2.16</td>
<td>1.26</td>
</tr>
<tr>
<td>Brisket 1.14</td>
<td>1.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cow #3160 - Milking when slaughtered 11/11 /64</th>
<th>Milk 9/64 contained 0.38 ppm. DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDE as DDT ppm.</td>
<td>DDT ppm.</td>
</tr>
<tr>
<td>Kidney 1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Omentum 1.24</td>
<td>1.22</td>
</tr>
<tr>
<td>Back 0.78</td>
<td>1.04</td>
</tr>
<tr>
<td>Round 1.40</td>
<td>1.48</td>
</tr>
<tr>
<td>Brisket 1.06</td>
<td>1.04</td>
</tr>
</tbody>
</table>
on the higher insecticide levels found in their milk during the test period. The method of analysis (performed at the Ithaca laboratory) was identical with that reported earlier for determining lindane in lamb tissues involving repetitive acetone-reflux extractions followed by hexane partitioning and electron affinity gas chromatography. The recovery of 2 ppm. of DDT and DDE from fat was 107, 137 and 84 and 70 percent, respectively.

The well-known persistence of DDT and metabolites is again demonstrated by the data presented here. More than six months was required to reduce excretion to low but still measurable levels. In November, 15 cows whose insecticide residue level has decreased below 0.05 ppm. were released from quarantine. The remaining animals were slaughtered for beef. Great financial losses or the end of a profitable business may well ensue during such a prolonged quarantine period. Even 10 months after withdrawal of the insecticide-contaminated feed, the body fat contained considerable residue so that continued excretion in the milk was to be expected, but at levels of DDT and DDE approaching the sensitivity of the analytical method. Body fat contamination at 10 months well under the 7 ppm. tolerance allowed.

ABSTRACT

A herd of 40 lactating cows which had been fed DDT-containing sweet corn silage were quarantined because of DDT residues in their milk. Disappearance of DDT in the milk was followed for ten months after cessation of silage feeding. DDT concentration decreased from 0.9 to 0.2 ppm. while DDE concentration remained quite constant at about 0.1 ppm. throughout this period. After ten months, fat from internal organs of sacrificed animals still contained up to 2.5 and 2 ppm. of DDE and DDT, respectively.

ACKNOWLEDGEMENT

The authors thank the New York Department of Agriculture and Markets for permission to publish their results in this paper.

REFERENCES CITED

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F. R. Koutz, Columbus, Ohio, Chairman; F. R. Biltovich, Baton Rouge, Louisiana; P. B. Doby, Springfield, Illinois; F. D. Enzie, Beltsville, Maryland; J. H. Galloway, Tempe, Arizona; H. J. Hourrigan, Hyattsville, Maryland; R. W. Kufrin, Benson, Minnesota; H. B. McGrath, Kansas City, Missouri; M. D. Mitchell, Pierre, South Dakota; R. D. Radeleff, Kierville, Texas; I. H. Roberts, Albuquerque, New Mexico; D. E. Worley, Bozeman, Montana

SHEEP SCABIES ERADICATION

Considerable Progress Made

The accelerated Sheep Scabies Eradication Program has enjoyed remarkable success since its beginning in August 1960. At that time, 1421 counties in 27 States were considered Sheep Scabies Free, an area of 44 counties in one State was the only area officially recognized as a Sheep Scabies Eradication Area, and 1689 counties in 23 States were classified as Sheep Scabies Infected Areas. Now there are 3054 counties in 49 States recognized as Sheep Scabies Free and 100 counties in two States designated as Sheep Scabies Eradication Areas. The classification of areas as infected only no longer exists as an active eradication program is underway in all previously Infected Areas.

On-Farm Inspections Materially Increased

Inspections of sheep in farm flocks reached 21,085,187 animals, 168 infected flocks were found in the entire country as compared to 126 the previous year. This increased effort revealed 35 infected flocks in areas classed as scabies free involving the States of California, Texas, Kansas, Nebraska, Wyoming, Minnesota, Illinois, Ohio, Virginia, Maryland, New Jersey, Pennsylvania and New York. Locating and eliminating these foci of infection contributed greatly toward the goal of complete eradication.

Additional Areas Become Scabies Free

During FY 1965 all or parts of the States of Nebraska, Missouri, Illinois, Indiana, Ohio, West Virginia, Kentucky and Tennessee achieved scabies-free status.

Greater Emphasis in Problem Areas

In order to step up work in areas which were delaying the eradication effort, two inspections of all sheep were made in the States of Nebraska, Indiana and Iowa. In the latter State, 119 outbreaks were thus disclosed. These inspections represented the first concentrated attack in recent years on the disease in Iowa.
Eradication of self-sustaining screwworm populations has been achieved in Arkansas, Louisiana, Oklahoma, New Mexico and Texas, and the program has been extended into Arizona and California.

A barrier zone has been established along the United States–Mexico border from the Gulf of Mexico to the Pacific Ocean.

The majority of the sterile screwworm flies produced at the laboratory in Mission, Texas, are being released in Northern Mexico to push screwworm populations as far south of the United States as possible. In spite of this drop in Northern Mexico, occasional gravid female flies manage to penetrate the sterile fly barrier zone and infest animals in Texas and New Mexico. As these sporadic outbreaks are found, emergency measures are taken each time to eliminate the infestations. In each case, the efforts have been successful and no self-sustaining population has become re-established.

A survey will be conducted throughout the Republic of Mexico to determine the location at which the barrier zone can be operated most efficiently.

In Fiscal year 1965 there were 6,292 laboratory-confirmed cases of screwworms reported from Mexico, and 2,406 cases in fiscal year 1964. A total of 613 laboratory-confirmed cases of screwworms was reported
from the United States in fiscal year 1965, as compared with 5,277 screwworm cases reported in fiscal year 1964. In Texas there were 343 laboratory-confirmed cases reported in fiscal year 1965, as opposed to 3,529 cases in fiscal year 1964. There were no screwworm cases reported in Oklahoma, Arkansas, and Louisiana in fiscal year 1965. By comparison, in fiscal year 1964, 18 cases were reported in Oklahoma and no cases in Louisiana and Arkansas. New Mexico's 39 laboratory-confirmed cases of screwworms in fiscal year 1965 contrast with the 1,102 cases reported in fiscal year 1964. Arizona's 231 cases reported in fiscal year 1965 is a decrease from the 540 cases which occurred in fiscal year 1964. Limited surveys in California revealed no screwworm cases in fiscal year 1965 and 88 cases in fiscal year 1964.

During fiscal year 1965, 4,557,703,214 sterile screwworm flies were released over the Southwestern United States and Northern Mexico. This compares with a total of 5,623,066,200 sterile screwworm flies released in fiscal year 1964.

CATTLE FEVER TICK ERADICATION

Prevention—keeping Boophilus annulatus, B. microplus, and other exotic ticks out of the United States is the major part of the effort against these ticks. A State and Federal quarantine zone including parts of eight southern Texas counties is maintained along the Rio Grande River as adjacent areas in Mexico are infested.

Active Program Continued in Texas

During fiscal year 1965, in the buffer zone 218 livestock illegally crossing the border were caught, of which 11 were tick-infested; 16 tick-infested United States herds were found; and 65,864 lots of 1,883,409 livestock were inspected for ticks and 13,219 lots of 85,165 livestock were dipped.

Tick Surveys

Emphasis on the importance of collecting and identifying ticks from all livestock species continued. During 1964 calendar year, 4,107 survey collections were made. This included 2,062 from cattle; 1,214 from horses; 408 from dogs; 289 from zoo animals and miscellaneous hosts; and 134 from native wildlife.

Ticks Found on Imported Animals and Materials

Collections from animals or materials imported into the United States included the following: Haemaphysalis wellingtoni, Haemaphysalis laeuchii leachii, Ixodes cookei, Amblyomma cyprium, A. rotundatum, A. dissimile, A. gemma, A. lepidum, and Rhipicephalus sanguineus. The latter tick is also found in the United States; however, those involved in the importation could well have been carriers of an exotic disease.
Four outbreaks of psoroptic cattle scabies were reported in four States. In California psorotptic mites were identified on March 10, 1965, from skin scrapings taken by a veterinary practitioner who was doing other professional work in a small herd of beef cattle in Kern County and suspected one steer had scabies. This was the first case reported in California since 1954 when outbreaks were also reported in Arizona, Colorado, Missouri, Oklahoma, and Texas.

On March 1965, a veterinary practitioner's report led to a diagnosis of scabies in a Wabaunsee County, Kansas, feedlot. This was the first evidence of psoroptic cattle scabies found in Kansas since 1959 when 13 infected herds were found in an outbreak involving nine counties.

A Meat Inspection Division veterinarian at a Federal establishment at Clovis, New Mexico, observed signs of scabies on March 30, 1965, affecting cattle from Castro County, Texas. Psoroptic mites were collected from the cattle at Clovis and also from cattle remaining on the premises in Texas. In April 1964 the disease had been diagnosed in cattle of the same owner. Three outbreaks of psoroptic cattle scabies were reported in Texas in 1962, one in 1961, two in 1959, one in 1956, three in 1955, and two in 1954.

In New York, on April 21, 1965, a regulatory veterinarian testing a Montgomery County dairy herd for tuberculosis observed scabies lesions. Psoroptic, chorioptic, and sarcoptic mites were identified from skin scrapings.

With the exception of the herd in New York, the outbreaks involved multiple possible sources and cattle shipped from several States. Although extensive epidemiological work was done it was not possible to pinpoint the source of any of the outbreaks. During the year, 18,389,099 cattle were inspected for scabies, an increase of approximately one million over the previous year.
TOXIC RESIDUES IN MEAT PRODUCTS

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Washington, D.C.

Today, the American housewife is deeply concerned about the wholesomeness of her meat, and the potential dangers of these and other unknown chemical compounds. She has read accounts of residues in foods, and has strongly expressed her demand that meat be free of injurious or unwholesome properties.

Yet, since she is more remotely removed from the source of her food, she must rely on others to determine its acceptability and wholesomeness.

Protecting her meat supply is the primary function of the Federal meat inspection service. It is just one of many programs administered by the U. S. Department of Agriculture's Consumer and Marketing Service which benefit not only the consumer, but also the producer and the entire industry. Grading, market news, regulation to insure fair play in the market, surplus removal, and consumer food programs are other C&MS services which help to assure the Nation of a dynamic food industry.

Each of us here today is well aware that miraculous technological developments have made it possible to greatly increase our supply of animal protein. What we may not be fully aware of, however, is the extent to which some of these developments rely upon chemical compounds which also can affect the wholesomeness of our food.

A few statistics on the production of red meat help to demonstrate this point. The consumption of red meat by the American consumer rose from approximately 145 pounds per person in 1945 to 174 pounds per person in 1964. During the same period, the American population rose from 140 million people to 192 million. Yet, how many are aware that in 1962 alone, 43 percent of the 44 million tons of complete feeds and supplements produced contained one or more drugs to increase their productive potential.

Today, pesticides are readily used to control plant and animal pests and disease to increase production. Animals are fed or injected with hormones or hormone-like substances and organic chemicals for more rapid meat development and more efficient feed conversion. And, animals are administered therapeutic drugs for the prevention and treatment of disease.

We can reasonably conclude that every meat animal in this country is produced in a constant environment of agricultural chemicals, and/or is administered one or more drugs during its lifetime.

The potential for chemical and drug residues in the edible tissues of food animals is therefore enormous. Thus, it is increasingly necessary to

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protect our food supply from adulteration with toxic or otherwise harmful residues.

To completely fulfill this vast responsibility, the meat inspection service must identify and eliminate potential dangers from toxic residues in meat. In the past, the primary job of the Federal meat inspector has been to see that bacteria and viruses were not introduced through meat examined and passed for food. We still have these intruders in food animals. In addition, we now must guard against the hazards of radioactive substances and chemical residues.

Viruses and bacteria leave telltale signs, but scars of the chemicals are not always as prominent. An otherwise healthy appearing animal may carry unsafe levels of residues, or have its true condition masked by the use of tranquilizers or antibiotics.

The meat inspection service has always recognized the potential dangers from toxic residues in meat. The 1906 regulations provided for the condemnation of animals showing the effects of lead, arsenic, mercury, and other intoxicants. With the increased use of agents which produce toxic residues in meat, the Division in 1949 initiated studies to detect and evaluate chlorinated-hydrocarbon pesticides in meat. This activity has been greatly expanded, and sensitive techniques have been developed to identify and determine levels of biologically-active residues.

The residues of greatest concern from a regulatory standpoint are those which produce an unwholesome condition in the meat or other edible parts of the animal. They generally result from compounds used as insecticides—for growth promotion—or for control of parasites and disease. These compounds are all chemicals and some have high biological potency. A few parts per million of some will produce a measurable biological change. It is obvious that misuse of these materials, either by accident or design, may produce a residue that is a hazard to the health of the consumer.

This hazard to health is often difficult to assess. We in meat inspection are particularly concerned with the effects of chronic toxicity. Though these effects have not been determined for any extended period, they can be inferred from the effects on laboratory animals fed at varying levels over the normal life span of the test animal. In areas where precise toxicological data are not available, a questioning attitude must be followed—not only for residues of the original chemical, but for those materials that in some instances are formed in the animal body from these substances. These metabolites may be toxic, perhaps even more toxic than the parent compound.

The permissible level of residue which can be considered safe is always in question. This question involves tolerances of residues. I do not intend to enter into the controversy involving residue tolerances. Being a regulatory function, the meat inspection service must follow the tolerances now established by law.

The important point is that the consumer must be told how little residue actually remains in the meat she buys. It is equally important for the producer and the veterinarian to understand the reason for the strict
conditions under which they both must operate to come within the limit of residue as required by law.

One part per million of residue is a surprisingly small quantity. Yet, in some cases, this minute quantity is highly biologically-active in the animal and its products. This biologic activity may cause physiological, pathological, pharmacological, and/or toxicological change in the animal. This is extremely important to the meat inspector, who relies heavily on abnormalities observed on ante-mortem inspection. Any one of these factors can mislead the veterinary inspector in judging the acceptability of an animal.

Time permits only a brief discussion of the residue findings and post-mortem conditions which our veterinarians have observed in animals slaughtered at Federally-inspected establishments. I have a few color slides which will better describe some of the adverse affects of drugs. Usually, the visible signs produced by such compounds stimulate the most interest and can best be understood. Unfortunately, this is not true in the case of insecticide residues.

Insecticides: Of the pesticide chemicals, the chlorinated-hydrocarbon insecticides present the greatest problem. Two characteristics that make this group more of a problem from a residue standpoint than the organic phosphates, are their tendency to accumulate in the fat and to persist. The organic phosphates, on the other hand, are rapidly eliminated.

Meat Inspection's residue surveillance program has revealed DDT, BHC, Lindane, Dieldrin, and Heptachlor Epoxide to be a problem in isolated herds. In a few of these herds the residue level was quite significant. These discoveries prompted others to carry out further investigations. The findings indicated that several situations were permitted which contributed to the tissue residues in the slaughter animals. The one major factor was a disregard for the labeling instructions. For instance, insecticides designed for plant application were applied directly to the animal. A restricted insecticide was used in a backrubber so the animal had continued exposure to the chemical. The animal was later presented for slaughter without regard to established withdrawal periods.

It has been demonstrated that animals with unacceptably high levels of residue in their tissues will gradually eliminate the residue if no further contamination occurs. Thus, the establishment of withdrawal periods before slaughter is justified in some cases. Many withdrawal periods from time-of-use to time-of-slaughter have been made a part of the label instructions on pesticides. The same is true with many of the medicated feeds. These withdrawal periods must be strictly observed.

Misuse of insecticides also occurs frequently when producers purchase such chemicals in small quantities, by filling their own containers from the bulk tanks of the distributor. Often times the producer doesn't label the container, nor does he bother to obtain proper information on the application of the chemical. The result often leads to over application, followed by residues in the meat of animals which consume the treated feeds.

We have other indications that chemicals affect wholesomeness by
causing odors in meat. As early as 1937, when Emmel and Henley reported on the effect of feeding paradichlorobenzene treated corn to swine, there were indications that chemicals can produce odor in meat. Feeding trials have been undertaken to determine if paradichlorobenzene would impart a flavor to the meat. It was found that feeding 0.95 lbs. of the chemical over a period of three weeks would result in a pronounced "moth ball" odor in the meat of the treated animal.

In fact here in New York some commercially-produced hog carcasses were found which contained odors and residues of paradichlorobenzene. They had been fed on home garbage. Many home deodorant products contain orthodichlorobenzene, crystals with paradichlorobenzene, and detergents.

Another example of pesticide residues occurred recently when a truck load of boneless beef was rejected because of odors caused by 2,4-D. The truck had transported the chemical before being loaded with meat. Even though the truck was thoroughly cleaned before the meat was loaded, the 2,4-D still persisted to an extent that it caused an odor in the meat.

There are many other causes of meat odors. Aromatic medicinal preparations administered to animals a short time before slaughter are sometimes detected during inspection, because of an odor imparted to the meat by the drug. Such meat is unacceptable for human food.

Medicated Feeds: All residue problems are not confined to insecticides. The first drug problem to reach serious proportions involved the use of hormone pellets implanted in feeder cattle and lambs. These pellets were detected by meat inspectors in subcutaneous tissues of the neck, back, and other locations of carcasses during routine post-mortem inspection.

Diethylstilbestrol, a synthetic compound with hormone-like properties, has become an essential part of many feeding operations. Feeds containing specified dose levels of this estrogenic hormone may be fed to cattle and sheep, provided the hormone is withdrawn from the animal at least 48 hours before slaughter to provide for elimination of the residues.

In some mammals, notably selected strains of mice, diethylstilbestrol has demonstrated carcinogenic properties. Carcinogenic action of estrogens has not been demonstrated in humans. But, one cannot be completely assured that the continued intake of synthetic estrogens by humans would not lead to malignant changes in susceptible tissues under heredity conditions. Consequently, animal tissue which contain any amount of diethylstilbestrol residue cannot be passed for human food.

There is nothing to indicate that a residue will remain, provided a 48 hour interval exists between the withdrawal of feed containing the estrogen and slaughter. The ideal additive would be one that could be fed to the animal right up to the time of slaughter without residues showing up in the edible tissues. Unfortunately, most authorized feed additives do not achieve such an objective. It is important to understand that the withdrawal periods have nothing to do with the effect of the additive on the animal. They have been established solely to protect the consumer.
We have also observed boar hogs in which diethylstilbestrol implants were indiscriminately used. The pellet was implanted behind the ear, a part of the animal normally used for food. The reason given for implanting the boars was to improve the texture and flavor of the meat. It goes without saying that practices of this kind will jeopardize the swine industry.

You may recall that a similar problem occurred many years ago involving chicken parts converted to mink food. In those cases, the hazard was to the mink, causing heavy economic losses to the producer and the poultry and fur industries.

Arsenic compounds have also been used in the past to improve health and appearance, and to stimulate growth in poultry and swine. Arsenilac acid, 3-Nitro-4-Hydroxyphenylarsonic acid, and sodium arsanilate are the most common added to feeds. Although these compounds were first noted for swine parasite control—and to a lesser extent disease control—they have been found to stimulate growth in swine and poultry very much as do the antibiotics.

The most commonly encountered residues in swine, from medicated feeds, are the organic arsenicals. The swine producer is required to withdraw his animals from arsenic feeds five days prior to slaughter. Investigations of herds in which significant arsenic residues have been found show that this warning was not always followed. Such residues are generally confined to the liver. Last week, I observed some hogs which had five times the recommended dose level of arsenic for a period of two weeks. These hogs already showed signs of arsenic poisoning.

**Therapeutic Drugs and Biologics:** The misuse of therapeutic drugs and biologics can have a long-lasting influence on the wholesomeness of meat from the treated animal. We have seen irritating lesions in the muscle of cattle and swine caused by tetracycline preparations. *(Slide 1 Swine muscle after antibiotic injection in axillary region) (Slide 2 Cattle muscle after antibiotic injection): (tetracycline)* *Slide 3 Tetracycline beef gluteal muscle.*

The increasing number of antibiotic lesions we see on post-mortem inspection indicates that more and more animals are being treated with drugs too close to slaughter. The evidence often shows up as an injection mark or pocket of antibiotic. The tissue assay may show residues only at the site of the lesion, or it may (if recently administered) be found in the liver, kidney, or muscle tissue other than at the site of injection. Assays have revealed penicillin, tetracyclines and sulfanamide.

The introduction of antibiotics into our meat source poses the same problem as does the indiscriminate use of other chemicals. The hazard in these cases is not one of direct toxicity. Rather, it stems from the possible development of antibiotic-resistant microorganisms, as well as the reaction in individuals who are highly sensitive to antibiotics.

The United States Department of Agriculture and the Department of Health, Education and Welfare are still seeking an answer as to the safety of an antibiotic-sensitive person who eats meat containing significant levels of antibiotic residues. Fortunately, antibiotics that have been
injected directly into the muscle of the food animal can usually be detected. These cases usually contain high levels of antibiotics at the site of injection. However, we occasionally find significant levels in other tissues of the animal's body. Our veterinarians, who perform a thorough post-mortem inspection on each animal slaughtered at a Federally-inspected establishment, have done an excellent job of sorting out those animals which exhibit tissue changes.

Drugs and biologics suspended in certain oily vehicles have produced granulomatous lesions. The next three slides show the resulting effects in cattle. (Slide 4 Bovine fat tissue-area of the round showing effects of vitamin injection.) (Slide 5 Bovine muscle—showing yellow granulomatous areas in the muscle.) (Slide 6 Experimental duplication in rat muscle.)

The effects of antithyroid compounds have produced enlarged thyroid glands in sheep. These compounds have been proposed for their ability to aid in fattening livestock. In addition to the lesions they may leave in the animal, these compounds leave residues to contaminate the carcass. The meat will be considered adulterated, unless the animals are allowed sufficient time to eliminate the chemical before slaughter.

Recently, enlarged thyroid glands were observed in experimental cattle which had been fed aminotriazole—the notorious weed-killer which gained fame during the cranberry episode a few years ago. This chemical produced hyperplasia and goiter in the thyroid, indicating that aminotriazole is also a goitrogenic substance. The activity of goitrogenic substances is not known, but some evidence indicates they prevent the proper utilization of iodine, which is essential in the formation of thyroxin. Smith and Jones (Veterinary Pathology) present evidence that the goitrogenic substances interfere with the production of thyroxin. Without going further into the physiological changes, it should be pointed out that thyroxin governs the rate of metabolism. Thyroxin also influences the nervous system, growth (especially that of the bones) and the mental and physical development of the animal.

Other pathological and physiological deviations affecting the wholesomeness of food animals have been observed by veterinary meat inspectors. Recently, we encountered an interesting problem—but one that could be very hazardous—involving fungicide-treated seed grain that was converted to animal feed. This is an illegal practice! Until more information is available on the effect of fungicides on the animal and residues in the tissues, the producer must take precautions not to feed grains containing fungicides. In many cases the animals died from eating treated grains before we were confronted with the question of residues.

Tranquilizers have caused problems when recommendations for their use were not followed. Regardless of the withdrawal period specified to assure residue elimination, no animal should be under the effects of a drug that is capable of masking symptoms of disease at the time of slaughter.

In the fall of 1963 and winter of 1964, meat packers suffered extensive economic losses from hams stained with injectable iron. Iron stained tissues have also been observed in beef carcasses. The condition
is characterized by a crownish-yellow discoloration several inches in diameter involving areas of muscle, fat, and connective tissue. We recognize that injectable iron has a definite place in the treatment of baby pig anemia. However, it is not advisable for mature animals, since many of the iron preparations persist for long periods of time. (Slide 8 Iron stained tissue and test reaction) Chemical tests for the presence of iron in tissue samples showed a positive reaction. Because of the economic importance and the effect on pork consumption, meat packers, the drug industry, the Food and Drug Administration, and others cooperated to reduce the losses caused by improper administration of injectable iron. So far this year, we have had few reports which confirm iron staining in meat. (Slide 9 Lipid granuloma—Hog shoulder vaccination lesions. Slide 10 Hog sl. abscess—vaccination crystal violet.)

Another area in which the veterinarian, researcher, and producer must be careful is that involving the slaughter of animals fed or treated with experimental preparations. Strict regulations must be satisfied before investigational animals are offered for slaughter. Part 309.20 of the Federal Meat Inspection regulations outlines the requirements for those engaged in experimentation who desire to salvage the animal for food.

Many aspects of biological residues need further research and investigation. Some have been explored from an economic standpoint without regard to the wholesomeness of the animal which ultimately will be offered for food. A few areas which we have under study at this time are: 1) Proposal for the feeding of poultry litter to food animals; 2) Residues which may occur in meat as a result of aerial application of undiluted concentrations of parathion on cotton acreage; and, 3) Forced anemia in calves to be slaughtered for human food. Regarding the latter, diets are used to accomplish this goal, and so are agents which bind the iron in the animal's body. Such agents are suspected of being in certain milk-replacer preparations.

In summation, the C&MS biological residue surveillance program indicates that most of the unsafe residues found in food animals are caused by the misuse of chemicals and/or drugs. This includes: (1) Slaughtering animals before the required withholding period elapses; (2) using drugs to mask pathological or physiological signs in the animal at time of slaughter; (3) feeding medicated feeds to animals for which they are not authorized; and (4) unintentional exposure of animals to chemicals that leave tissue residues. We recognize that the proper use of these substances is essential in maintaining and increasing our meat production. We realize also that each chemical and drug serves a distinct function—and that there is a correct and incorrect way to use each substance to fulfill its function.

The problems that arise from the use of residue-producing insecticides and drugs are naturally viewed with widespread concern by the public health veterinarian. Complex controls over chemicals that may get into our meat supply through a biological process are therefore required. It becomes the responsibility of the entire veterinary medical profession to develop the competency to cope with such residues, and to assure every consumer that meat is a safe and wholesome commodity.
Agriculture, since the time of recorded history, has encountered many difficulties in adapting to changes brought on by the advancement of technology. Of primary concern today are the public criticisms relevant to the use of drugs and chemicals and the allegations of their detriment to man's socio-economic well-being. It is ironic that a public could be so aroused by Silent Spring and yet be so complacent about the automobile, for it too has the "power to kill" and an "incredible potential for harm." Psychologically it is easier to precipitate fear of the things we do not understand.

Rapid scientific advances of science have made available a vast armamentarium of drugs and chemicals of which the general public has little knowledge.

In like manner, the rapid advances in agriculture are beyond the comprehension of a predominately urban population. The ever present abundant supply of quality fruits, vegetables and meats detracts further from the consumers appreciation of the role that drugs and chemicals play in making this possible.

It is also ironic that penicillin was alluded to as the "wonder drug" and heralded as major scientific achievement; whereas the scientific accomplishments in relation to pesticides and certain other drugs are identified primarily as public or social hazards without recognition of the great public benefit from proper use. Yet anyone knowledgeable in this field appreciates that penicillin improperly used has the ability to produce harm. Thus the key term in relation to this situation is the word "improper." This was graphically alluded to in an editorial appearing in the September 1965 Canadian Journal of Comparative Medicine and Veterinary Science as follows: "Electricity improperly used can be lethal, yet no one in his right mind would suggest that we limit the expanding applications of electrical power. The answer to the "Pesticide problem" if there is such a thing, is surely greater government awareness of the necessity of entrusting the use of potentially dangerous chemicals to those who are qualified by training to use them properly."

Of equal concern is the "No-Residue" and "Zero-Tolerance" concepts for pesticides in the United States. This is reflected in the Report of the Pesticide Residue Committee of the National Academy of Sciences. The report, released on July 19, 1965, contained eleven recommendations. A synopsis of these are as follows: The concepts of "no-residue and zero-tolerance" currently employed administratively are untenable and should
be on the basis of either "negligible residue" or "permissible residue." Where use may result in a residue in or on food, registration should not be granted unless levels are within "negligible" or "permissible" standards. When registration is on a negligible-residue basis, the negligible-residue figure should be published together with the analytical method for determining the excess of the negligible residue. The amount and analytical method should have Food and Drug Administration concurrence and be controlling for enforcement purposes. The FDA regulations on permissible residues should include a published description of the analytical methods used for enforcement and not changed without notice and opportunity for comment by those interested. A pesticide known to be too hazardous for a particular use should not be registered. Because of their relationship to food production and nonfood uses incident to the national health and economy, pesticide registration should remain the responsibility of the U.S. Department of Agriculture. Publish a reasonable schedule for the orderly transition from present procedures, and its duration decided by mutual agreement between the Departments of Agriculture and Health, Education, and Welfare. Develop programs for continuing centralized leadership, exchange of information, training activities, and interlaboratory evaluation together with a manual on residue methods. A formal program for education in residue analysis cooperatively sponsored by concerned agencies at suitable training centers. Expand research on persistence of pesticides in the total environment, thru toxicological, pharmacological, and biochemical studies to improve reliability and precision of animal studies and their relevance to man. Pharmaceutical means for the destruction of agricultural pests is at a high level of effectiveness. However, this Committee feels that research should be encouraged and intensified toward utilizing and improving biologic control methods.

The improper use of certain drugs as feed additives and for therapeutic purposes may be considered analogous to the "pesticide problem." Through such improper use residues can and do result. This is evidenced by the monitoring program of Meat Inspection Service.

The manufacturers of drugs must establish safety and efficacy standards before drugs are approved for use. This information is translated so that the directions for use are clearly stated. It will then behoove the user, be he veterinarian or farmer, to respect and comply with these provisions in the interest of animal and public health.
THE RESISTANCE AND CARRIER STATUS OF CHICKENS EXPOSED TO MYCOPLASMA GALLISEPTICUM UNDER FIELD CONDITIONS*

T. H. Vardaman, DVM, M.S.
Athens, Georgia

During the past few years interest has been shown in the effects of vaccination or early exposure of chicks to Mycoplasma gallisepticum and its subsequent effect on egg transmission and immunity.

Intramuscular injection of M. gallisepticum organisms into immature chicks produces some immunity. Intranasal injection of M. gallisepticum has been shown to produce some resistance to challenge. The injection of a highly pathogenic strain of M. gallisepticum into the posterior thoracic air sacs of immature chickens produced resistance to challenge. The birds did not shed M. gallisepticum in the eggs before or after challenge, while in the control negative birds after the same challenge, 70.9 percent of the eggs were infected with M. gallisepticum.

Recently there has been widespread inoculation of immature breeder birds with M. gallisepticum in many of the poultry areas of the United States. However, there is a lack of adequate evaluation of egg transmission of M. gallisepticum in field-exposed birds.

The purpose of this study was to determine the efficacy of the exposure of immature chicks to M. gallisepticum to control egg transmission and to determine if the controlled exposed birds would transmit the disease to normal birds during the laying period.

MATERIALS AND METHODS

A commercial flock of six thousand 40-day-old White Rock chickens were injected intranasally with 0.1 ml doses of a broth culture of R strain M. gallisepticum supplied by the University of Georgia Poultry Disease Research Center. At 23 weeks of age, five males and 50 females were selected at random and transferred to the Southeast Poultry Research Laboratory (SEPRL) and placed in separate isolation units. Five males and 30 females of the same breed and age, grown at SEPRL and maintained free of M. gallisepticum were used in the studies.

The media used were: Difco Brain Heart Infusion broth (BHI), Difco PPLO broth, and viande foie (VF). Each broth medium was dispensed at three ml. per tube. The solid media were the same with the addition of 1.4 percent agar. All media were supplemented with 0.5 percent yeast autolysate (Albimi), 0.0005% thiamin hydrochloride, 0.3 percent Tris

*From the Southeast Poultry Research Laboratory, Animal Disease and Parasite Research Division, Agriculture Research Service, U.S. Department of Agriculture, Athens, Georgia. Dr. Vardaman is now at the South Central Poultry Research Laboratory, State College, Mississippi. The author wishes to thank Mr. Boyd Turner, Medical Biological Technician, for his assistance.
buffer, 0.025 percent thallium acetate, 0.3 percent dextrose, 0.05 percent BBL Trypticase, 0.0025 percent phenol red, 10.0 percent heat inactivated swine serum, and 1000 units penicillin per ml.

At 27 weeks of age all birds were tested for pullorum agglutinins by the tube agglutination test, and for *M. gallisepticum* agglutinins by the serum plate (SP) and hemagglutination inhibition (HI) tests. At that time, tracheal swabs from all birds were cultured for *M. gallisepticum*. Each tracheal swab was placed in BHI broth, incubated at 37°C, and the broth observed daily for three weeks. When acid formation was evident, 0.5 ml. of the culture was transferred to BHI broth, PPLO broth, VF broth, and one drop to each plate of the three solid media. The broth cultures were observed daily and, if acid formation were observed, the cultures were frozen for later hemagglutination (HA) tests. On the fourth and seventh day of incubation the solid cultures were microscopically examined at 80X magnification for Mycoplasma colonies.

At 28 weeks of age, the birds were divided into the following groups, each group maintained in separate isolation, and treated as follows:

**Group 1.** 10 *M. gallisepticum*-positive (vaccinated at 40 days) females and two males. (Used as vaccinated positive controls.)

**Group 2.** 10 *M. gallisepticum*-positive (vaccinated at 40 days) females and two males. Females were challenged intranasally with 0.2 ml. and intratracheally with 0.2 ml. of a 1:100 dilution of egg yolk R strain (homologous challenge) *M. gallisepticum*. This R strain egg yolk material had an embryo lethal dose (ELD)₅₀ of 10⁶.₈ per ml.

**Group 3.** Same as Group 2 except females were challenged with V strain (heterologous) *M. gallisepticum*. The V strain egg yolk material had an ELD₅₀ of 10⁴.₃ per ml.

**Group 4.** 10 *M. gallisepticum*-free females and 2 males. The females were challenged intranasally with 0.2 ml. and intratracheally with 0.2 ml. of a 1:100 dilution of egg yolk R strain *M. gallisepticum*. (Used as non-vaccinated positive controls).

**Group 5.** 10 *M. gallisepticum*-free females and one male (used as normal controls).

**Group 6A.** 20 *M. gallisepticum*-positive females (vaccinated at 40 days) were placed with Group 6B.

**Group 6B.** 10 *M. gallisepticum*-free females and two males (used as contact controls).

All birds were bled at five, 10, 16 and 22 weeks post challenge date and *M. gallisepticum* serum plate tested and the remaining serum was frozen at -65°C. The serum from these four bleedings was *M. gallisepticum* HI tested at one time using 4 HA units antigen and 0.25 percent washed chicken red blood cells.

Tracheal swabs were taken from all birds at five, 10 and 16 weeks post challenge. Each tracheal swab was placed in BHI broth medium, incubated at 37°C, and observed daily. If an acid reaction was observed, a portion of the culture was filtered through a sterile 0.45 or 0.8 micron
porosity Millipore* filter. Subsequently one drop of filtrate was placed on BHI solid medium and approximately 0.5 ml. added to a tube of BHI broth medium and incubated at 37°C. The liquid and solid cultures were observed and treated as explained above.

Beginning one week prior to challenge and for four weeks post challenge, all eggs were trapnested, identified individually, incubated for 18 days and attempts made to isolate *M. gallisepticum* from the yolk and its membranes using the same three-broth media used for isolations from the tracheas of the adult hens.

Beginning the fifth week after challenge and continuing for three weeks, eggs were collected and identified by groups and stored at 7°C. All eggs were placed in the incubator on the same date, hatched and chicks from each group reared in batteries in isolation until nine weeks old. All chicks were inoculated intraocularly with B1 strain of Newcastle disease vaccine at three weeks of age and intraocularly with infectious bronchitis vaccine at five weeks of age. Chicks that died were cultured for *M. gallisepticum*. At nine weeks of age, each chick was bled, killed and examined for lesions. Serum from each chick was tested for *M. gallisepticum* by the serum plate test.

Beginning the eighth week after challenge and for seven consecutive weeks, eggs were collected daily by groups and stored at 7°C. Each Monday, the eggs were placed in the incubator and candled on the seventh, 14th and 18th days. After hatching each day-old chick was bled, killed, and checked for lesions. Attempts to isolate *M. gallisepticum* were made from portions of the trachea taken at the bifurcation near the lung, placed in BHI broth medium and incubated at 37°C. Isolation attempts were made from yolks of dead embryos. The same culture procedure was used as described above.

Representative isolates from the yolk of 18-day embryos and from tracheas of day-old chicks were injected into seven-day embryonated eggs known to be free of *M. gallisepticum* to check their pathogenicity. Eggs that died from the 4th to the 12th day post injection were considered to be caused by *M. gallisepticum*. The yolk of eggs that died were cultured for *M. gallisepticum*. Embryos that did not die were allowed to hatch and were checked for lesions and isolation attempts were made for *M. gallisepticum*.

RESULTS AND DISCUSSION

I. Adult Birds

The results of tracheal swab culture for *M. gallisepticum* are shown in Table I. At one week pre-challenge *M. gallisepticum* isolations were made from 40.0 to 60.0 percent of the birds in groups vaccinated (1, 2, 3 and 6A) and none from the nonvaccinated groups (4, 5, and 6B). Even though 1000 units of penicillin per ml. and 0.025 percent thallium acetate were used in the media to prevent contamination, there was some

*Millipore Filter Corporation, Bedford, Massachusetts.*
TABLE I

Results of Tracheal Isolations of *Mycoplasma gallisepticum* from Adult Birds

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Pre-Challenge</th>
<th>Post Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 wk. Contamination</td>
<td>5 wks.</td>
</tr>
<tr>
<td>Group 1</td>
<td>4/6*</td>
<td>9/9</td>
</tr>
<tr>
<td>Group 2</td>
<td>6/8</td>
<td>10/10</td>
</tr>
<tr>
<td>Group 3</td>
<td>5/8</td>
<td>9/10</td>
</tr>
<tr>
<td>Group 4</td>
<td>0/5</td>
<td>2/10</td>
</tr>
<tr>
<td>Group 5</td>
<td>0/2</td>
<td>0/10</td>
</tr>
<tr>
<td>Group 6A</td>
<td>10/13</td>
<td>17/18</td>
</tr>
<tr>
<td>Group 6B</td>
<td>0/6</td>
<td>9/10</td>
</tr>
</tbody>
</table>

* = Numerator = number of isolations obtained

Demonominator = number of isolation attempts made.

contamination in each group. At five, 10, and 16 weeks post challenge, when sterile Millipore filters were used, there were from 80.0 to 100.0 percent isolations of *M. gallisepticum* from the tracheas of birds in: (1) group 1 and 6A that were vaccinated at 40 days, (2) groups 2 and 3 that were vaccinated at 40 days of age and challenged intranasally and intratracheally with a virulent strain of *M. gallisepticum* at 28 weeks, (3) and from group 6B, normal birds that had been in contact for five weeks or longer with birds that were vaccinated at 40 days with *M. gallisepticum*. All tracheal isolates hemagglutinated chicken red blood cells, which is typical of *M. gallisepticum*. The isolation of *M. gallisepticum* from the tracheas of approximately one-year-old birds exposed while young confirms the work of Olson, *et al.*

The higher percentages of *M. gallisepticum* tracheal isolations on five, 10, and 16 week post challenge date were apparently due to the use of sterile Millipore filters. Sterile Millipore filters with 0.45 or 0.8 micron porosity will filter out bacterial contaminants and permit passage of *M. gallisepticum* organisms. Tourtellotte, *et al.* described the use of these filters in primary isolation of *Mycoplasma*.

From group 4, negative birds that were injected with a virulent culture of *M. gallisepticum* at 28 weeks of age, there were 20.0, 44.4 and 33.0 percent *M. gallisepticum* isolations at five, 10 and 16 weeks post injection, respectively. All the birds in group 4 showed severe respiratory symptoms 10 to 30 days post injection. The low percentages of *M. gallisepticum* isolations from the tracheas of birds in group 4 may be due to the birds being 28 weeks old when vaccinated. Little difference was noted in respiratory symptoms or tracheal isolations from birds in group 2 and 3 after challenge at 28 weeks with 0.4 ml. of a 1:100 dilution of egg yolk R strain (homologous) and V strain (heterologous) *M. gallisepticum*, respectively.

Birds in groups 2 and 3 showed only mild respiratory symptoms in contrast to severe respiratory symptoms in birds in group 4, thus indicating some resistance to challenge in those birds vaccinated at 40 days...
of age. Other workers have shown some resistance to immunization of immature chicks.\textsuperscript{1,2,3,4}

On pre-challenge bleedings, all birds that were vaccinated with \textit{M. gallisepticum} at 40 days of age were positive to the \textit{M. gallisepticum} serum plate and HI tests. All pre-inoculation control birds, grown at SEPRL, were negative to both tests.

There were no significant differences in the rise in HI titers of birds in vaccinated groups 2 and 3 that were challenged at 28 weeks with a virulent culture of \textit{M. gallisepticum} when compared with vaccinated groups 1 and 6A that were not challenged.

Normal birds in group 4, that were injected at 28 weeks with \textit{M. gallisepticum} gave higher HI titers at five, 10, 16 and 22 weeks post-injection when compared with birds from vaccinated groups 2 and 3 that were challenged, and vaccinated groups 1 and 6A that were not challenged.

The birds of group 6B, at five weeks post contact with birds vaccinated at 40 days with \textit{M. gallisepticum} (group 6A) were negative to both \textit{M. gallisepticum} SP and HI tests; at 10 weeks post contact, only one bird (10.0 percent) was positive to SP test, but at 16 and 22 weeks post contact, 60.0 percent and 100.0 percent of the birds respectively were positive to SP and HI tests. Even though \textit{M. gallisepticum} was isolated from 90.0 percent of the tracheas from birds in group 6B at five weeks post contact with birds in 6A, and was isolated from 100.0 percent at 10 and 16 weeks post contact, only one bird (10.0 percent) was positive to the SP test at 10 weeks post contact. There were slight respiratory signs in some of the contact birds in group 6B. These results are essentially the same as those of Olesiuk and Van Roeke\textsuperscript{6} who reported that after three months cohabitation with serological positive birds the susceptible birds will essentially all become serologically positive and that the transmission occurred with only slight clinical evidence of the disease in a few birds.

\section*{II. Porigeny of Adult Birds}

The number of \textit{M. gallisepticum} isolates from the yolk of 18 day-old embryos and from the tracheas of one day-old chicks are presented in Table II.

The reason for the relatively low percentage of \textit{M. gallisepticum} isolations from the yolk of groups 2, 3 and 4 may be due in part to the fact that eggs were cultured for \textit{M. gallisepticum} for only 28 days after birds were inoculated. Up to 28 days post injection, the birds were probably not shedding enough \textit{M. gallisepticum} organisms via egg for isolations with the limited number attempted. However, all isolates from the yolk were from eggs that had been stored at 70 C. for 24 hours or less.

Although there were no lesions found in the day-old chicks, \textit{M. gallisepticum} was isolated from tracheas of chicks from groups which had been vaccinated only, and from vaccinated groups which were subsequently challenged. Chicks from contact control group apparently were not infected at this time.

\textit{M. gallisepticum} broth culture isolates from these groups hemagglutinated chicken red blood cells and were lethal when inoculated into
the yolk of seven-day embryos which is typical of \textit{M. gallisepticum}. Embryos that did not die were allowed to hatch and were examined for lesions. These chicks showed stunting, deformities and air sac lesions. \textit{M. gallisepticum} were re-isolated from the yolk of the dead embryos and from air sac lesions of each chick as determined by microscopic examination of the colonies grown on solid medium. Serum samples collected from the day-old chicks each week were sampled by the serum plate test. The percent positive declined from 60.0 percent to 25.0 percent over a period of seven weeks for groups 1, 2, 3 and 6A. The percent positive for group 4 declined from 100.0 percent to 60.0 percent over the same period. All chicks from birds in groups 5 and 6A were negative to the serum plate test.

The results of the progeny birds of each group reared to nine weeks are shown in Table III. \textit{M. gallisepticum} isolations were attempted on all

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
\textbf{Group No.} & \textbf{No.} & \textbf{9 wk.} & \textbf{1st week} & \textbf{Other} & \textbf{Slightly Cloudy air sacs} \\
\hline
Group 1 & 76 & 70 & 3* & 3 & 0 \\
Group 2 & 101 & 96 & 5 & 0 & 1 \\
Group 3 & 71 & 66 & 4 & 1 & 0 \\
Group 4 & 37 & 34 & 2 & 1 & 3 \\
Group 5 & 30 & 27 & 3 & 0 & 0 \\
Group 6A & 77 & 60 & 14 & 3** & 9 \\
Group 6B & 14 & 12 & 2 & 0 & 0 \\
\hline
\end{tabular}
\caption{Results of Progeny Birds Reared to Nine Weeks}
\end{table}

\*\textit{M. gallisepticum} was isolated from one bird that died at five days.
\**One bird at six weeks was a runt, was injured, sacrificed, had lung lesions, \textit{M. gallisepticum} isolation was negative.
chicks that died. The majority of the deaths occurred during the first week. Only one *M. gallisepticum* isolation was made from dead chicks. It was from a chick in group 1 that died at five days of age. One bird from group 6A was injured and was killed at six weeks. On examination lung lesions were present, but *M. gallisepticum* was not isolated. The serum from this bird was negative to the SP test.

On post mortem examination at nine weeks, slightly cloudy air sacs were found in groups 2, 4 and 6A. However, the lesions observed were not severe enough to cause the birds to be condemned. Sera from all of the birds were negative to the SP test. Since each group of chicks was reared in isolation under controlled laboratory conditions in small batteries, the possibility of spread of *M. gallisepticum* by contact was limited.

The eggs used for producing the progeny grown to nine weeks of age were stored at 70°C from 0 to 20 days prior to incubation. It is possible that prolonged storage of *M. gallisepticum*-infected eggs at low temperature may reduce or eliminate egg transmission. Further research is needed on the effect of storage on egg transmission of *M. gallisepticum*.

**SUMMARY**

Birds injected intranasally at 40 days of age with 0.1 ml. of a broth culture of the R strain of *M. gallisepticum* were shedding *M. gallisepticum* organisms from 27 to 44 weeks of age as shown by tracheal isolations and spread of the organisms to susceptible birds.

The progeny of vaccinated birds were not free of *M. gallisepticum* since isolations were made from the yolk of 18-day embryonated eggs and from the tracheas of day-old chicks.

While *M. gallisepticum* was present at one day of age from all progeny groups, except 5 and 6B, the techniques employed failed to demonstrate the presence of *M. gallisepticum* at nine weeks of age.

It is suggested that negative results in nine week-old progeny may be attributed to prolonged storage of hatching eggs at 70°C prior to incubation.

**REFERENCES CITED**


A NEED FOR PULLORUM-TYPHOID ERADICATION

A. E. Janawicz
Montpelier, Vermont

Forty years ago pullorum disease was a serious menace to our poultry industry. The extensive and marked infections in our poultry stocks exerted a retarding influence on the growth of the industry. However, through scientific investigations of the disease, scientists discovered and developed effective methods to combat the malady. State programs for the testing of breeding flocks were initiated in a number of states during the twenties. The promise of this testing work was soon recognized which encouraged the New England States to organize a conference for the standardization of techniques and to adopt eradication of the disease as their primary goal for future years. Much has been accomplished by this organization which in subsequent years expanded to include 14 Northeastern States and two Canadian Provinces.

In 1935, the National Poultry Improvement Plan was introduced. The development of the whole blood test and the introduction of a national voluntary pullorum control program should be credited with the marked reduction of infection in poultry flocks as observed today. However, the greatest credit for eliminating the disease from our flocks should go to the breeders, hatcherymen, and growers. Without their cooperation the marked reduction in the infection level never could have been attained. Credit should also be given to the state and federal agencies that rendered effective service in administering the testing programs.

The progress made in reducing the infection from breeding flocks is most impressive. In Table I is listed a thirty-seven year summary (including the first and last years of the period) for 14 states and two Canadian provinces. This table reveals the growth in the industry and marked reduction in the percentage of infection. One also notes the decrease in the number of "breaks" and the marked increase in the number of pullorum-clean birds.

<table>
<thead>
<tr>
<th>Item</th>
<th>1927-28*</th>
<th>1963-64</th>
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<tbody>
<tr>
<td>No. of flocks</td>
<td>1,349</td>
<td>3,677</td>
</tr>
<tr>
<td>No. of birds</td>
<td>694,468</td>
<td>11,519,932</td>
</tr>
<tr>
<td>Positive tests (based on birds) (%)</td>
<td>5.05</td>
<td>0.004</td>
</tr>
<tr>
<td>No. of negative flocks (100% tested)</td>
<td>372</td>
<td>3,617</td>
</tr>
<tr>
<td>No. of &quot;breaks&quot; in negative flocks (100% tested)</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td>No. of birds in pullorum-clean flocks</td>
<td>112,605</td>
<td>11,239,137</td>
</tr>
</tbody>
</table>

*Does not include North Carolina, Vermont, and Virginia.
Table II lists the progress made by each of the 14 states and two Canadian provinces in the Northeastern Conference on Avian Diseases. In 1964, a total of six states revealed no reactors among tested flocks.

Table III lists the number of cases of pullorum disease and fowl typhoid detected in the testing of breeding flocks and in the diagnostic laboratories (as reported by the 14 states and two Canadian Provinces). It should be emphasized that pullorum infection can be detected from time to

<table>
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<tr>
<th>State</th>
<th>Year</th>
<th>Birds tested</th>
<th>Percentage positive</th>
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<tbody>
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<td>20,743</td>
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<tr>
<td></td>
<td>1964</td>
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<tr>
<td>Delaware</td>
<td>1925</td>
<td>4,300</td>
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<td></td>
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<td>Maine</td>
<td>1921</td>
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<td>New York</td>
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<td>59,576</td>
<td>6.20</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>400,092</td>
<td>0.00</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1932</td>
<td>64,702</td>
<td>4.02</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>4,683,635</td>
<td>0.002</td>
</tr>
<tr>
<td>Nova Scotia</td>
<td>1929</td>
<td>2,041</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>76,394</td>
<td>0.06</td>
</tr>
<tr>
<td>Ontario</td>
<td>1928</td>
<td>15,000</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>1,220,296</td>
<td>0.005</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1924</td>
<td>2,077</td>
<td>15.00</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>1,274,591</td>
<td>0.00008</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>1925</td>
<td>8,175</td>
<td>6.97</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>43,091</td>
<td>0.00</td>
</tr>
<tr>
<td>Vermont</td>
<td>1928</td>
<td>8,555</td>
<td>7.40</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>78,523</td>
<td>0.00</td>
</tr>
<tr>
<td>Virginia</td>
<td>1925</td>
<td>13,000</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>1,110,708</td>
<td>0.0086</td>
</tr>
<tr>
<td>West Virginia</td>
<td>1928</td>
<td>9,005</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>40,561</td>
<td>0.0024</td>
</tr>
</tbody>
</table>
A NEED FOR PULLORUM-TYPHOID ERADICATION 313

TABLE III

Follow-up of Cases of Pullorum Disease and Fowl Typhoid (1963)*

<table>
<thead>
<tr>
<th>Tested flocks</th>
<th>Diagnostic Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pullorum</td>
</tr>
<tr>
<td>Retested</td>
<td>17</td>
</tr>
<tr>
<td>Retested and partial depopulation</td>
<td>1</td>
</tr>
<tr>
<td>Total depopulation</td>
<td>4</td>
</tr>
<tr>
<td>Incomplete eradication</td>
<td>-</td>
</tr>
<tr>
<td>No follow-up</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
</tr>
</tbody>
</table>

*Data submitted by fourteen Northeastern States and two Canadian Provinces.

time in essentially all states in this area. Attention is called to the follow-up on these outbreaks. Infection detected among tested flocks was followed up in all instances, but concerning outbreaks detected by the diagnostic service, an appreciable number of infections were either not investigated nor eliminated.

On a national level progress in eradication is also impressive. Table IV reveals the progress that has been made during a 15-year period. The majority of birds tested are classified as pullorum clean.

TABLE IV

Progress in Pullorum Disease Eradication for a Fifteen-Year Period in the United States

<table>
<thead>
<tr>
<th>Item</th>
<th>1950</th>
<th>1956</th>
<th>1964</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flocks</td>
<td>111,422</td>
<td>70,468</td>
<td>18,816</td>
</tr>
<tr>
<td>No. of birds</td>
<td>37,237,674</td>
<td>36,112,781</td>
<td>36,257,094</td>
</tr>
<tr>
<td>Percent reactors</td>
<td>0.72</td>
<td>0.07</td>
<td>0.006</td>
</tr>
<tr>
<td>Birds in clean flocks</td>
<td>13,302,642</td>
<td>31,273,701</td>
<td>34,421,844</td>
</tr>
</tbody>
</table>

In regard to pullorum-typhoid isolations, the results are listed in Table V. The Western Region had the smallest number of cases, whereas the South Atlantic Area had the largest. However, in comparing these regional results one must take into account the poultry population as well as the type of industry that prevails in the area. It is again clearly evident that in each section pullorum infection still exists.

In areas such as the New England States, as well as some other states, further progress in eradication cannot be expected unless more effective measures are adopted to prevent the introduction of the infection from out-of-state sources. In Table VI are listed six consecutive years of testing for the 14 states showing the number of years no reactors were found. These results reveal that some states have a long series of
TABLE V
Pullorum-Typhoid Isolations (1963)*
(Figures in parentheses indicate number of follow-up investigations reported)

<table>
<thead>
<tr>
<th>Region</th>
<th>0-5 mo.</th>
<th>Over 5 mo.</th>
<th>Age not reported</th>
<th>Total</th>
<th>Follow-up Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Atlantic</td>
<td>14</td>
<td>13</td>
<td>0</td>
<td>27</td>
<td>70.37</td>
</tr>
<tr>
<td>E. No. Central</td>
<td>13</td>
<td>24</td>
<td>3</td>
<td>40</td>
<td>55.00</td>
</tr>
<tr>
<td>W. No. Central</td>
<td>48</td>
<td>33</td>
<td>0</td>
<td>81</td>
<td>8.64</td>
</tr>
<tr>
<td>So. Atlantic</td>
<td>44</td>
<td>59</td>
<td>1</td>
<td>104</td>
<td>64.42</td>
</tr>
<tr>
<td>So. Central</td>
<td>38</td>
<td>18</td>
<td>2</td>
<td>58</td>
<td>37.93</td>
</tr>
<tr>
<td>Western</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>18</td>
<td>61.11</td>
</tr>
<tr>
<td>United States</td>
<td>163</td>
<td>155</td>
<td>10</td>
<td>328</td>
<td>45.12</td>
</tr>
</tbody>
</table>


TABLE IV
Six-Year Summary of the Incidence of Pullorum Reactors in Fourteen Northeastern States

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Conn.</td>
<td>0.004</td>
<td>0.006</td>
<td>0.004</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Del.</td>
<td>0.0006</td>
<td>0.009</td>
<td>0.107</td>
<td>0.107</td>
<td>0.00</td>
<td>0.0009</td>
</tr>
<tr>
<td>Maine</td>
<td>0.0021</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0002</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Md.</td>
<td>0.016</td>
<td>0.019</td>
<td>0.008</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0009</td>
</tr>
<tr>
<td>Mass.</td>
<td>0.0001</td>
<td>0.001</td>
<td>0.0034</td>
<td>0.0003</td>
<td>0.00</td>
<td>0.0002</td>
</tr>
<tr>
<td>N. H.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.073</td>
</tr>
<tr>
<td>N. J.</td>
<td>0.02</td>
<td>0.003</td>
<td>0.0065</td>
<td>0.001</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>N. Y.</td>
<td>0.0002</td>
<td>0.00</td>
<td>0.0038</td>
<td>0.0006</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>N. C.</td>
<td>0.007</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.0006</td>
<td>0.002</td>
</tr>
<tr>
<td>Pa.</td>
<td>0.09</td>
<td>0.02</td>
<td>0.02</td>
<td>0.018</td>
<td>0.0008</td>
<td>0.00008</td>
</tr>
<tr>
<td>R. I.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vt.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.008</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Va.</td>
<td>0.0226</td>
<td>0.029</td>
<td>0.0268</td>
<td>0.0249</td>
<td>0.006</td>
<td>0.0086</td>
</tr>
<tr>
<td>W. Va.</td>
<td>0.0045</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0094</td>
<td>0.00</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

negative tests while in others the results may be positive on a persistent or intermittent basis. Concerning "breaks" that have appeared, a trace back on the histories in most cases revealed that persons who purchased the stock have either totally disregarded checking on the pullorum-clean status of the stock or just assumed that the stock was pullorum clean. On the other hand, some breeders, hatcherymen, or chick handlers have
knowingly sold stock that was suspicious of the disease. As long as these practices are permitted to prevail, the disease will not be eliminated.

Furthermore, every focus should be eliminated as soon as detected and its origin should be determined, and if found, should likewise be eliminated. The logic and necessity for complete follow-up is obvious if the disease is to be eliminated. Until such a program is instituted together with more effective measures to prohibit the sale and purchase of questionable or infected stock, we may expect outbreaks of pullorum disease to appear. The National Poultry Improvement Plan which is a voluntary plan for participants is inadequate to cope with all possible disease situations that can be expected to arise. Further, being a voluntary plan it does not require that all breeding flocks, large and small, including fancier and backyard flocks, be tested. Birds exhibited at fairs and shows should come from only clean flocks. The testing results in some areas have revealed that pullorum infection is largely detected in scattered fancier flocks while commercial breeding flocks are essentially negative. Until an official regulatory program for the eradication of pullorum disease is instituted, further progress in eliminating the disease appears doubtful.

The purpose of eradicating pullorum disease is not primarily for the sake of eliminating the disease but to free the poultry industry of this malady and to eventually eliminate the expense incurred through testing, medication, and other miscellaneous items. The eradication results to date have shown convincingly that the disease is very amenable to eradication. This can be explained by a study of the ecology of the organism. The only obstacle in the path toward eradication is the attitude of man regarding the problem. Some in the poultry industry are fearful that regulatory programs and agents will interfere with their inherent rights. Many states have in operation a disease regulatory program that is designed for livestock, including poultry. Competent personnel are employed to implement the control and eradication programs. Likewise, the Federal Government, through its federal-state cooperative eradication programs, has competent personnel to deal with these disease problems. In some states the state and federal personnel are already actively engaged in the control and eradication of pullorum disease and fowl typhoid.

Furthermore, the Federal Government and state animal disease regulatory agencies have a cooperative reporting system that reveals the incidence of certain diseases by states. Every state should regard pullorum disease and fowl typhoid as reportable diseases, making it mandatory that these diseases be reported to the state animal disease regulatory agency. At the present time, the lists that have been reported may be regarded as incomplete, unreliable, and restricted. If a disease is to be eradicated we must have full knowledge of its existence.

At the 1964 meeting of the United States Livestock Sanitary Association, Uniform Methods and Rules for area eradication of pullorum disease and fowl typhoid were approved and submitted to the Animal Health Division, Agricultural Research Service, United States Department of Agriculture, for consideration. The Animal Health Division feels that these Uniform Methods and Rules constitute a technically sound approach for
the eradication of pullorum disease and fowl typhoid and recommends them to states wishing to initiate such a program.

Each state regulatory agency should review this program with its industry members. It should consider if any legislation is needed to effectively implement the program. With appropriate legislation and effective administration of the program, pullorum disease should be eradicated completely so that at some time in the future flock owners will receive some relief from the cost of annual testing. The success of eradication will in a large measure depend upon the support and cooperation the industry will give toward completely eliminating the disease.

It is our sincere belief that the time is here to start thinking in terms of eradication in lieu of control.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

H. E. Goldstein, Columbus, Ohio, Chairman; R. A. Bankowski, Davis, California; F. G. Buzzell, Augusta, Maine; H. L. Chute, Orono, Maine; G. E. Coleman, Jr., Brunswick, Maine; L. C. Grumbles, College Station, Texas; R. Houge, Lafayette, Indiana; T. L. Landers, Jefferson City, Missouri; G. L. McNeilly, Brookings, South Dakota; R. L. Ricker, Dover, Delaware; J. R. E. Taylor, DeKalb, Illinois

The Transmissible Diseases of Poultry Committee this year has continued the effort of initiating a national eradication program for Pullorum Disease and Fowl Typhoid.

A meeting was called for October 25, 1965 of the General Conference Committee of the National Poultry Improvement Plans, the Eradication Committee of the American Avian Pathologist Association, and the Committee of Transmissible Diseases of Poultry for the United States Livestock Association.

This meeting afforded all three groups a better understanding of the problems and ramifications involved in initiating a national eradication program. Industry participants indicated an interest in the eradication of Pullorum Disease and Fowl Typhoid but were most emphatic in pointing out more serious and economic problems.

A subcommittee for the eradication of Pullorum Disease and Fowl Typhoid was appointed this year. Dr. Henry Van Roekel was chairman of this subcommittee. Dr. Van Roekel is retiring this year from the University of Massachusetts. Dr. Van Roekel has dedicated many years of sincere effort in poultry disease programs, both in research and laboratory service. He has been one of the most enthusiastic leaders of our group for eradication of Pullorum Disease and Fowl Typhoid.

The subcommittee for the eradication of Pullorum Disease and Fowl Typhoid submitted the following report:

During the past year a subcommittee of the Committee on Transmissible Diseases of Poultry was established to enhance the eradication of pullorum disease and fowl typhoid. A committee of five members was appointed representing federal and state regulatory personnel and state experiment station workers. The primary attention of this committee was directed largely toward seeking more detailed and reliable information regarding the present status of these diseases and to stimulate further interest in their eradication.

On February 4, 1965 the Animal Disease Eradication Division distributed a Memorandum No. 512.4 to all its Animal Disease Eradication stations which introduced the Uniform Methods and Rules for Area Eradication of Pullorum Disease and Fowl Typhoid as approved by the United States Livestock Sanitary Association at its 1964 Annual Meeting. This
program was recommended to the states wishing to initiate such a pro-
gram. The Animal Disease Eradication veterinarian in charge was re-
quested to review the Memorandum with the responsible state official.

Members of the Committee have had an opportunity to participate in
regional meetings to further the eradication of pullorum disease and fowl
typhoid. However, a critical evaluation of the information presented at
these regional meetings of workers in pullorum disease control and
eradication has revealed that there are numerous aspects of the present
National Plans program that appear incompatible with enhancing the
eradication of these two diseases. The primary weakness in the present
program is that the control and eradication of the disease is voluntary for
the participants. Furthermore, in many states the regulatory agency ex-
ercists no control over the program. Diagnostic laboratories do not feel
duty bound to report outbreaks of these diseases to the agency that has no
regulatory power. Less than 50 percent of the known foci of these dis-
eases are followed up in the various states, and the voluntary aspect of
the Plan precludes the use of adequate authority and power to enforce
regulations that are necessary ingredients for an effective eradication
program. The lack of or the mixed responsibilities by state animal dis-
ease regulatory officials for the control and eradication of these diseases
are serving as a deterrent to effective eradication.

During 1964, a total of 33,175,336 chickens was tested which revealed
.0044 percent reactors. Also, 3,798,357 turkeys were tested with .0002
percent reactors.

Recently, the Committee circulated a brief questionnaire to regula-
tory officials in the respective states to seek certain information that
might appraise the situation more realistically. Out of 50 questionnaires
distributed, 37 were returned. The questionnaires revealed the following
information:

1. Number of states having an official pullorum-typhoid -
   a. Testing program 32
   b. No program 5

2. Jurisdiction of the Program under -
   a. State animal disease regulatory agency 21
   b. Other agency in the state Department of Agriculture 3
   c. Special poultry industry board 6
   d. State Experiment Station or similar agency 3

3. The testing of flocks is subject to regulatory enforcements by -
   a. State animal disease regulatory agency 22
   b. Other agencies 5
   c. No enforcement 5

4. Number of states with laws applicable to pullorum-Typhoid
   control 22
TRANSMISSIBLE DISEASES OF POULTRY

5. Number of states with mandatory testing of all commercial breeding flocks - 12
6. Number of states permitting sale of only pullorum-clean stock within the state - 12
7. Number of states permitting the importation of only pullorum-clean stock - 22
8. Number of states in which pullorum disease and fowl typhoid are reportable diseases - 32
9. Number of states in which animal disease regulatory agency allocates funds to pullorum disease control - 18
10. Number of states favoring the proposed area eradication program adopted by the U. S. Livestock Sanitary Association 31
11. Pullorum-fowl typhoid incidence (1964) -
   a. Total cases of pullorum disease detected in tested flocks - 196
   b. Total cases of fowl typhoid detected in tested flocks - 54
   c. Total cases of pullorum disease detected in diagnostic laboratories - 175
   d. Total cases of fowl typhoid detected in diagnostic laboratories - 91
   e. Total cases of pullorum disease reported - 371
   f. Total cases of fowl typhoid reported - 145
      516

The information furnished by the various states reveals that an appreciable number of states have laws or regulations that can be applied to pullorum disease and fowl typhoid control. On the other hand, in only a few states is the testing of all commercial breeding flocks mandatory. Also, only a few states permit the sale within the state of only pullorum-clean stock. However, in regard to importation of stock, 22 states specified that such stock must be pullorum-clean. Pullorum disease and fowl typhoid are reportable diseases in most states.

As one analyzes the collected information for the different states, one is impressed that many of the states have recognized and adopted some of the important ingredients that are necessary for a sound eradication program. However, there are certain aspects in an appreciable number of state programs that must be strengthened in order that a more effective program may be developed.

Your Committee wishes to enumerate the following recommendations for your consideration:

1. Each state should strive to delegate the jurisdiction of the pullorum-typhoid control work to its animal disease regulatory agency.
2. All commercial breeding flocks should be required to be pullorum-clean.
3. Only pullorum-clean stock should be sold within the state or imported from out-of-state.
4. Pullorum disease and fowl typhoid should be reportable diseases and all infections should be reported to the animal disease regulatory agency.
5. All known pullorum disease and fowl typhoid infections should be eliminated under the supervision of the regulatory agency.
6. All birds exhibited at shows or fairs should come from pullorum-fowl typhoid-clean flocks.
7. A feasible and reliable reporting system on these diseases should be developed and their incidence listed by states. These disease reports should be channeled through the state disease regulatory agency to the Director of the Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
8. This subcommittee should be continued but its membership should be increased.

Last year's Committee requested the Minnesota program for the control and eradication of *Mycoplasma gallisepticum* in turkeys be submitted for Committee consideration.

A copy of the following was presented by Dr. J. G. Flint, Secretary and Executive Officer for the Minnesota State Livestock Board and Dr. Werring, Veterinarian in Charge, United States Department of Agriculture, Agriculture Research Service, Animal National Health Division.

**SUGGESTED RULES AND REGULATIONS FOR THE CONTROL AND ERADICATION OF MYCOPLASMA GALLISEPTICUM IN TURKEYS**

**GENERAL PROVISIONS**

1. The *Mycoplasma gallisepticum* control program shall be administered on a voluntary basis and any flock, hatchery or hatching egg dealer may participate providing it complies with the following procedures:

   a. Files a signed agreement with the State Livestock Sanitary Official, and complies with these rules and regulations.
   b. Has not violated the terms of the above signed agreement or these rules and regulations, resulting in cancellation until such time has elapsed as the State Livestock Sanitary Official shall consider sufficient for reinstatement.
   c. When more than one hatchery located within the State is operated under the same name, ownership or management, one or more of these hatcheries shall not participate in the *Mycoplasma gallisepticum* control program unless all participate. All such hatcheries shall attain and maintain the same classification.
TRANSMISSIBLE DISEASES OF POULTRY

TESTING

1. All turkeys four months of age and over to be used for breeding purposes and participating under these regulations, shall be tested for *Mycoplasma gallisepticum* using the same blood sample collected for conducting and pullorum, typhoid and typhimurium tests. Breeders shall not be selected from flocks having a history of, or showing symptoms of a respiratory infection which is known to be egg transmitted. A turkey breeding flock held over for a second season's production shall be considered a new flock and tested accordingly.

2. All tests shall be conducted with an antigen approved by the State Livestock Sanitary Official, and such tests with all antigens shall be considered when final determination for a flock or hatchery classification is made.

3. Reactors as designated by the official laboratory shall be presented for necropsy and bacteriological examination to an official laboratory within 10 days from date of test, and accompanied by a shipping permit on which is recorded the individual band numbers. The following criteria shall be used to determine if the flock can receive an official classification:
   
a. Active air sac lesions.
   
b. Recovery of *Mycoplasma gallisepticum* organisms.
   
c. Supplemental serological tests.

4. Flocks which contain birds that are serologically positive, but show no active air sac lesions on necropsy and *Mycoplasma gallisepticum* is not recovered, shall be considered suspicious. A one hundred (100) random sample test will be conducted on such flocks prior to the time they come into production. The final determination will be based on the same criteria as outlined above in subparagraph 3, or a negative random sample test will qualify the flock. Additional tests shall be conducted if deemed necessary.

CLASSIFICATION

1. A *Mycoplasma gallisepticum* tested flock is a flock which, when officially tested for *Mycoplasma gallisepticum* in an official laboratory under supervision of the State Livestock Sanitary Official, contained no reactors to the *Mycoplasma gallisepticum* antigen, or a suspicious flock that is eventually declared to have no reactors after additional tests, and bacteriological examinations are made. The qualifying test shall be made within six (6) months prior to first sale of hatching eggs.
   
a. The above classification shall not be issued to any flock with clinical signs or symptoms of infectious sinusitis.
   
b. Only birds of the same or comparable classification may be added to a *Mycoplasma gallisepticum* tested flock.
c. Poults and hatching eggs originating from *Mycoplasma gallisepticum* tested flocks may receive the same classification provided they are handled, hatched and reared separate and apart from other hatching eggs or poults not so classified.

2. A *Mycoplasma gallisepticum* tested hatchery is one operating under the supervision of the State Livestock Sanitary Official, and with the exceptions provided for in the following paragraph, hatching and handling only eggs and poults originating from *Mycoplasma gallisepticum* tested flocks or from flocks of comparable status.

3. If facilities satisfactory to the State Livestock Sanitary Official for complete segregation are available, eggs from flocks not under this program or eggs from other species of poultry may be incubated and the products from such incubated eggs hatched and brooded in a *Mycoplasma gallisepticum* Tested Hatchery provided; such eggs are incubated and hatched in separate machines, and the products of such eggs are maintained in complete segregation from the poults hatched from eggs originating in a *Mycoplasma gallisepticum* Tested flocks. A thorough cleaning, disinfection and fumigation program shall be conducted on all equipment and hatchery prior to setting other eggs.

**HATCHERIES AND FLOCKOWNERS**

1. The hatchery management shall:
   a. Permit inspection of buildings, equipment and poultry products contained therein at any reasonable time by agents of the State Livestock Sanitary Official.
   b. Maintain identity of hatching eggs as to flock and place of origin.
   c. Keep hatchery and incubator room well isolated from battery room.
   d. Practice recommended procedures for fumigation of incubators and hatchers.
   e. Use only new egg cases or used egg cases that are clean and have been fumigated between each use.
   f. Maintain available and adequate records to show origin of all hatching eggs and destination of poults sold for the current year and one year previous.

2. The flockowner shall:
   a. Maintain poultry buildings and premises in a sanitary condition.
   b. Permit inspection of flock and premises at any reasonable time by agents of the State Livestock Sanitary Official, and submit flock for collection of additional blood samples if deemed necessary.
   c. Attempt to eliminate animal disease carriers such as rats and mice, and discourage the presence of free flying birds.
d. Avoid raising other poultry and farm animals on premises unless well segregated.
e. Report immediately to a disease control official when any respiratory symptoms appear in the flock.
f. Refrain from using any drug that will mask the results of serological tests or bacteriological recovery for

ADVERTISING

1. The advertiser shall use only the classification which his birds, flocks or hatchery have attained under these rules and regulations.
2. All advertising shall specify the disease tested for, prefacing the word "Tested" with "Mycoplasma gallisepticum."

NON-PARTICIPANT

1. Products produced under these regulations shall lose their identity when purchased for resale or consigned to a non-participant.
2. A non-participant may not use the official terminology or any portion thereof of the Mycoplasma gallisepticum control program.

SUGGESTIONS FOR SANITATION AND MANAGEMENT PRACTICES IN BREEDER FLOCKS

1. Maintain breeder flocks on farms separate from grower flocks.
2. Avoid the introduction of new breeding stock unless the birds can be adequately segregated for 30 days, or until proved to be free of Mycoplasma organisms.
3. Prevent transmission from outside sources by indirect contact through contaminated equipment, footwear, clothing, and vehicles.
4. Provide adequate isolation for breeder flocks to avoid air-borne transmission from other flocks.
5. Minimize contact of breeder flock with game and free-flying birds.
7. Keep the rodent population and other pests under control.
8. Initiate a vaccination program for other poultry diseases aimed at the needs of the farm and area.
9. Allow no visitors to turkey houses or range premises.
10. Clean and disinfect all equipment after each use.
11. Maintain a footbath with an approved disinfectant.
12. Clean and disinfect confinement house or pole barn between blocks.
13. Use clean and well-drained areas for range.
14. Use clean dry litter free of mold.
15. Keep accurate records of death losses.
16. Dispose of all dead birds properly and promptly.
17. Seek services of a veterinary diagnostician if abnormal losses or signs of disease occur.
The Committee submits this program for review of the Association. This year's Committee revised the standard methods and rules for the eradication of pullorum disease and fowl typhoid. This revision was carried out to correct misunderstanding and to provide greater clarity.

The Uniform Rules and Methods should serve as a complement to existing state NPIP and NTIP programs. The Committee urges that, wherever possible, these suggestions as outlined be included in the rules of the NPIP and NTIP programs.

There are six important points to consider in implementing an eradication program for Pullorum Disease and Fowl Typhoid.

1. 100 percent participation of eligible flocks and hatcheries.
2. All laboratory and field diagnosis of the two diseases reported.
3. Proper procedures for handling and disposition of infected flocks and their products.
4. Testing required for all exhibition birds.
5. Requirements for importation.
6. Immediate follow-up of reported outbreaks.

The changes in the Uniform Methods and Rules were only changes in terminology and did not alter the intent so we will not take the time to read them. The changes will appear in the proceedings.

The Committee recommends that all state livestock sanitary officials consider including tests for pullorum disease and fowl typhoid for all poultry within their respective states. A negative flock requirement or an individual negative test will strengthen existing control programs and may well point out existing foci of infection assisting over-all eradication.

The Committee recommends that the subcommittee for the eradication of Pullorum Disease and Fowl Typhoid be continued in the Transmissible Disease of Poultry Committee.

The Committee discourages states to initiate or strengthen regulations governing intrastate movement of hatchery waste, diseased poultry, and offal.

The Committee recommends that the Transmissible Diseases of Poultry Committee and the United States Department of Agriculture Agricultural Research Service prepare a brochure on pullorum disease and fowl typhoid for national distribution to all facets of the industry involved.

Your Committee directs your attention to the serious problem of para-typhoid and Arizona infections of poultry and turkeys. Poultry and Turkey industry leaders report to your Committee that these diseases are causing heavy economic losses among their flocks. These leaders suggest that the United States Livestock Sanitary Association should take steps to provide assistance to the industry to reduce these losses. The industry leaders point out help is needed in the form of field studies and epidemiology to provide more information concerning the pathogenesis of these diseases which could provide the necessary tools in reducing the economic losses experienced each year.

Your Committee wishes to call your attention to the lack of adequate
service to the private and public laboratories servicing the animal and livestock industries for the sero typing of salmonella isolations from non-human sources. The Committee commends the service provided to date by the Animal Health Division, Agricultural Research Service, United States Department of Agriculture, at the National Animal Disease Laboratory at Ames, Iowa, but due to the apparent increasing workload, steps should be taken to expand this much needed service.

The Committee on Transmissible Diseases of Poultry is submitting a resolution to the Resolution Committee, that the president of the United States Livestock Sanitary Association consider the appointment of a Committee to be concerned with all aspects of the salmonella problem. This Committee should investigate all of the ramifications of salmonellosis and submit material and information for other specie committees to program.
RECOMMENDED PROCEDURES FOR THE ISOLATION OF SALMONELLA ORGANISMS FROM ANIMALS

L. C. Grumbles, College Station, Texas, Chairman; H. Van Roekel, Amherst, Massachusetts; M. M. Galton, Atlanta, Georgia; B. S. Pomeroy, St. Paul, Minnesota; E. M. Ellis, Ames, Iowa; Wilson Henderson, Lafayette, Indiana

INTRODUCTION

The isolation of Salmonella from animal tissues is not a single exact procedure that can be outlined in complete detail. Considerable judgment of a person who has knowledge of these infections in various animals and knows microbiologic procedures is essential. When the purpose of examination is to make a diagnosis on either an individual animal or herd or flock basis, careful judgment is necessary to properly interpret the isolation of a Salmonella.

The animal species and type of sample or specimen available often dictate different initial isolation procedures. While many Salmonella have a wide host range, some have a definite host preference. This should be considered when examining tissues from the various animal species. In general, however, the cultural procedures, biochemical characterization and serologic typing procedures are similar irrespective of the source of the Salmonella.

SELECTED PROCEDURES BASED ON ANIMAL SPECIES OR SPECIAL PURPOSES

Avian

Salmonella pullorum and Salmonella gallinarum (active infection)

1. Culture heart blood, liver and spleen directly to tryptose agar and to SS agar or brilliant green agar if desired. Inoculation of tetrathionate or selenite broth with subsequent plating on selective media may also be done. Culture five or more birds if available.
2. Incubate 18 to 24 hours at 37°C.
3. Select tryptose agar cultures having only one type colony (characteristic of Salmonella) or isolated colonies from selective media and transfer with standard bacteriologic loop to tubes of dextrose, maltose, lactose, sucrose and mannitol and inoculate into semi-solid agar to test for motility. Most types of S. pullorum produce acid and gas in dextrose and mannitol but do not attack maltose, lactose or sucrose. Occasional isolates of S. pullorum may produce acid in maltose.

S. gallinarum produces acid but no gas in dextrose, maltose and mannitol but does not attack lactose or sucrose. Dulcitol which is fermented by S. gallinarum but not by S. pullorum may be included to further assist in differentiation of S. pullorum and S. gallinarum. Any culture that
appears to be *S. pullorum* or *S. gallinarum* should be typed with salmonella "O" antiserum and should be in Group D.

**CULTURE OR Reactors TO CONFIRM SEROLOGIC TESTS**

An excellent outline of the recommended procedures are given in the National Poultry and Turkey Improvement Plans and Auxiliary Provisions (Miscellaneous Publication No. 739, ARS, USDA).

These procedures should be used in official control programs. In general, the procedure includes culturing various internal organs and intestinal content being especially careful to include the ovaries, gall bladder and any organ having lesions. The organs are cultured individually or as a composite sample by placing a portion of the material in tetra-thionate or selenite broth. After 18 to 24 hours incubation, the tetra-thionate or selenite broth culture is plated to brilliant green or SS agar. Identify as outlined for *S. pullorum* and *S. gallinarum*.

For the isolation of the paratyphoid organisms from avian species, see outline under General Procedures.

**Swine**

*Salmonella cholerae-suis*—this organism is not limited to, but is a frequent infection in swine. The procedures outlined apply to the isolation of the organism from all species. With the exception of enrichment, procedures for the isolation of *S. cholerae-suis* are the same as for most other salmonella.

Tetra-thionate and selenite broth are apparently toxic to *S. cholerae-suis* and should not be used as the only enrichment broth for isolating this organism.

Intestinal contents should be plated directly to brilliant green, SS and bismuth sulfite agar plates. Nutrient broth with plating to brilliant green or SS agar may be superior to direct plating. Identifying biochemical characteristics are given in Table I. The American variety of *S. cholerae-suis* does not produce H₂S, but the more common Kunzendorf variety does. Both varieties produce acid and gas from maltose, xylose and sorbitol, but do not attack trehalose, arabinose or inositol. Identify serologically as indicated under *Identification*, No. 3.

For isolation of other Salmonella in swine, see outline under General Procedures:

**Equine**

*Salmonella abortus equi*—this organism is almost entirely host specific; the horse is the susceptible animal.

1. Culture placenta, affected fetus (particularly stomach fluid) or uterine exudate into tetra-thionate broth and directly to tryptose agar slants and to brilliant green or SS agar.

2. Incubate 18-24 hours at 37°C and streak from tetra-thionate broth to selective media (brilliant green or SS agar plates). Select isolated,


### TABLE I

Typical Fermentation Reaction of Salmonella in Selected Carbohydrate* Media

<table>
<thead>
<tr>
<th>Organism</th>
<th>Dextrose</th>
<th>Mal-</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Salcin</th>
<th>Sorbitol</th>
<th>Trehalose</th>
<th>Dulcitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pullorum</td>
<td>AG</td>
<td>NV(v)</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>AG</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>S. gallinarum</td>
<td>A</td>
<td>A</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>S. cholera-suis</td>
<td>AG</td>
<td>AG</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>AG</td>
<td>NC</td>
<td>AG</td>
</tr>
<tr>
<td>S. abortus-equii</td>
<td>AG</td>
<td>AG</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>V</td>
<td>AG</td>
<td>V</td>
</tr>
<tr>
<td>(Paratyphoids)</td>
<td>AG</td>
<td>AG</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td>Arizona</td>
<td>AG</td>
<td>AG</td>
<td>Late</td>
<td>NC</td>
<td>NC</td>
<td>AG</td>
<td>AG</td>
<td>NC</td>
</tr>
</tbody>
</table>

*Most carbohydrate media can be purchased as a complete dehydrated medium containing the carbohydrate and phenol red indicator or the carbohydrate may be added to a phenol red broth base. It should be made up in six to 10 ml amounts in suitable tubes containing inverted Durham vials.

A - Acid; AG - Acid and Gas; V - Variable; NC - No Change.

typical colonies from the plates streaked from tetrathionate broth or directly with the sample and proceed to identify by carbohydrate fermentation and other biochemical tests (See Table I). Confirm serologically as indicated under Identification, No. 3.

### GENERAL PROCEDURES

Isolation of all *Salmonella spp.* other than those listed as having a preferred host.

*From All Animal Species*

Recommended procedures for the isolation of *Salmonella spp.* from feed and meat products have been described. (Recommended Procedures for the Isolation of Salmonella Organisms from Animal Feeds and Meat By-Products. ARS 91-36, October 1962, Published by Agricultural Research Services, USDA). Much of the procedure included in that publication is applicable to isolation and identification of Salmonella organisms from any source.

**Isolation Procedures**

1. Fecal samples—usually a sample of fecal material or a cotton swab representing a sample of rectal material taken from the live, intact host.
   
   (a) Place the swab or two to three grams of the fecal material in eight to 10 ml of tetrathionate broth. If sufficient fecal sample is available, place 30 grams in 100 ml of the broth or culture any given amount of the sample in this ratio to the broth.
   
   (b) Shake vigorously, then incubate for 18 to 24 hours at 37°C.
   
   (c) Plate to brilliant green or SS agar using standard bacteriological loop. Streak plate in a manner so that individual colonies will be
obtained. It may be advisable to dilute the tetrathionate broth in sterile water or saline to obtain a 1:100 and 1:1000 dilution before streaking the agar plates.

2. Animal tissues—when there is a possibility that Salmonella infections are systemic or involve organs other than the digestive tract, it is important to have selected organs and tissues for examination.

(a) When intact carcass or organs are available, make cultures direct to blood agar, tryptose agar, brilliant green agar or SS agar or some other combination of universal and selective media. Take specimen by use of bacteriological loop, sterile cotton swab, pipette or by macerating small portions of the tissues to be cultured. Culture various organs separately.

(b) Select a small portion of several visceral organs (liver, spleen, kidneys) and grind or macerate, then inoculate tetrathionate broth, incubate for 18 to 24 hours, then streak on brilliant green or SS agar.

Identification

1. Select typical colonies from brilliant green, SS or other agar cultured and inoculate triple sugar iron agar; stab the butt and streak the slant. This medium indicates ability to ferment lactose, sucrose and dextrose and the ability of the organism to form gas and hydrogen sulfide.

2. If reaction on triple sugar iron is typical for Salmonella (no change in slant and yellow or black butt) transfer to tubes of carbohydrate media (see Table I for reaction), lactose, dextrose, sucrose, salicin and dulcitol should be included along with other selected carbohydrates to help identify and characterize. Urease and indol production along with the inoculation of lysine iron agar and KCN medium may be helpful.

3. Remove growth from triple sugar iron agar after 18 to 24 hours incubation (after transfers to carbohydrate or other media for biochemical tests have been made) and suspend in physiological saline. Check this suspension with salmonella polyvalent "H" serum. If it reacts with the polyvalent serum it should be further checked with "D" group antiserum.

If the organism produces typical colonies on selective media, a typical reaction on triple sugar iron and in tubes of carbohydrate media, reacts with the salmonella polyvalent and one or more group antiserum, it may tentatively be considered a Salmonella spp. Identification to species should be accomplished by submitting the culture to the Salmonella Typing Center, National Animal Disease Laboratory, Ames, Iowa.

When a culture does not behave in a typical manner, it should be streaked again on a nutrient agar medium or MacConkey's agar and individual colonies selected and identified to assure that it is a pure culture.

Further biochemical characterization may include inoculation of the following media: urea agar, Simmons citrate agar, lysine decarboxylase broth and KCN medium. It may also be helpful to determine the morphology and gram staining characteristics by applying the standard Gram's

*Sets of Salmonella typing antisera available from Difco Labs., Detroit, Michigan.
staining technique. Determination of motility may also be useful. This can be done by direct examination of an unstained drop of the culture under high power magnification of the microscope or by the inoculation of semi-solid agar.

ADDENDUM

Most of the media suggested for use can be obtained as dehydrated media. They should be carefully prepared, stored, and used according to the manufacturer's instructions. Two good sources of such media are Difco Labs., Detroit, Michigan, and Baltimore Biological Laboratories, Baltimore Maryland. Other sources may be equally satisfactory.

Several media and procedures are described in the appendix of ARS 91-36 RECOMMENDED PROCEDURES FOR THE ISOLATION OF SALMONELLA ORGANISMS FROM ANIMAL FEEDS AND MEAT BY-PRODUCTS, October 1962. This publication should be used as a companion guide for the procedures suggested here, thus, the information given in ARS 91-36 are not repeated.
REPORT OF THE COMMITTEE ON PUBLIC HEALTH
AND RADIOLOGICAL FALLOUT

R. H. Huffaker, Atlanta, Georgia, Chairman; P. J. Brandly, Hyattsville, Maryland; R. D. Courter, Atlanta, Georgia; C. Pilch, Hazzardville, Connecticut; H. J. Rollins, Raleigh, North Carolina; I. M. Saturen, Ames, Iowa; R. J. Schroeder, Los Angeles, California; J. H. Steele, Atlanta, Georgia; F. A. Todd, Arlington, Virginia; F. B. Trum, Sherborn, Massachusetts; E. E. Wedman, Ames, Iowa; R. D. Wenger, Alexandria, Virginia; A. H. Wolff, Washington, D. C.

SALMONELLOSIS - A PROGRESS REPORT

During the past year, a number of steps have been taken by the Public Health Service and other Federal and State agencies and by the poultry and egg industries towards the elimination of salmonellae in egg products. Industry cooperation has been excellent.

The United States Department of Agriculture adopted regulations requiring, as of June 1, 1965, that all liquid eggs, regardless of whether they are to be distributed in the liquid, frozen or dried form, must be pasteurized or examined for the presence of salmonellae by a recognized laboratory method. When salmonellae are recovered, the product must be pasteurized before being released for consumption. These regulations require that by January 1, 1966, all egg products, except whites, must be pasteurized and that by June 1, 1966, all egg products, including whites, must be pasteurized. The pasteurization temperature required for liquid eggs is to be not less than 140°F for three and one half minutes. For liquid whites, a minimum temperature of 132°F for two minutes is required.

In the August 21, 1965, issue of the Federal Register, the Food and Drug Administration proposed to amend definitions and standards of identity for liquid eggs and liquid egg products, either frozen or dried, to require these articles of food to be pasteurized or otherwise treated so as to destroy all viable salmonella micro-organisms. These amendments will become effective October 21, 1965, if no objections to the proposals are filed.

Utah has passed a law requiring all egg products (liquid, frozen and dried) sold within the State, to be pasteurized. A law was passed this summer in California requiring pasteurization of egg products beginning June 1, 1966. The California law also prohibits the use of incubator rejects, dirty eggs and leakers in the preparation of liquid egg products. In addition, Colorado has adopted the United States Department of Agriculture regulations covering the grading and inspection of edible egg products. The Washington State Health Department is sponsoring efforts to secure legislation to require pasteurization of egg products.

It has been shown that certain salmonellae will survive the times and temperatures (132°F for two minutes or 140°F for three and one half
minutes) required for pasteurization of egg products in the United States regulations (Angelotti, et al.). Survival of salmonellae was demonstrated when the source of a number of cases of salmonellosis was found to be United States Department of Agriculture inspected and passed, pasteurized, dried canned whole egg solids distributed in Washington State (reported to the CDC Veterinary Public Health Laboratory by Dr. Bernard Bucove, Director of Health, Washington State Department of Health, July 1965). Although the current regulations are a step forward in control efforts, a temperature and time should be required which will kill all salmonellae. 1480 F. for two and one half minutes has been shown effective in the United Kingdom (Murdock, et al.). In addition, some measure of the effectiveness of the pasteurization procedure should be required, either cultural examination for salmonellae or the alpha amylase test.

During the past year, the Poultry and Egg National Board established a Salmonella Committee to determine ways to disseminate information to industry and to the consumer from various groups investigating the salmonella problem. This Committee, composed of poultry and egg industry representatives and poultry scientists, met in September 1964 and in January 1965 at the Communicable Disease Center with members of the Center staff concerned with salmonellosis. Representatives of the feed industry attended the January meeting. During these sessions, the Committee agreed to: (1) develop standards for shell eggs designed to produce salmonella-free eggs so that they can be safely used in hospitals and institutions; (2) promote effective pasteurization of all liquid egg products; (3) promote establishment of Federally approved poultry inspection in plants not presently under inspection; (4) stimulate feed producers to apply measures to eliminate salmonellae in animal feeds.

An ad hoc committee on salmonellae in eggs composed of a group of egg and cake mix industry representatives, established a sub-committee made up of the laboratory directors from their companies and a representative of the Public Health Service. This subcommittee developed methods for sampling "eggs, egg products, and prepared foods that contain eggs" and recommended adoption of specific laboratory methods for the detection of salmonellae in egg products and foods. These recommended procedures will be submitted for publication in Food Technology.

Leaders of the poultry and egg industry have been most cooperative during investigations concerning salmonellosis. In addition, they have sought information concerning control measures and bacteriological monitoring procedures for salmonellae. One national baby food processor has written into their specifications for purchase of poultry meat the requirement that it be examined for the presence of salmonellae and found negative. Before acceptance, this product also is examined for salmonellae in their own plant laboratory.

The Federal Food, Drug and Cosmetic Act provides authority for control of salmonella in food products, including animal feeds, in interstate commerce. While salmonellae are not identified specifically by name, section 402(a) of this Act defines a food as adulterated if it bears or contains any poisonous or deleterious substance which may render it
injurious to health. The Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, in cooperation with other Federal agencies and industry representatives, has prepared sanitary guidelines for processors of animal and poultry by-products and of fish meal used in feeds. If implemented, these procedures would help to reduce salmonella contamination in processed animal by-products used in feeds. These guidelines could also be developed into a sanitary code.

Encouraging results in processing animal feeds to eliminate salmonellae were reported at a meeting of the Nutritional Council of the American Feed Manufacturers Association this year. Among these was a report that when ground feed was inoculated with 50-80 million salmonellae per gram and processed by "pelleting," involving temperatures of 160° to 180° F. for from two to 16 seconds, some salmonellae survived, but when processed by the "expansion-extrusion" method in which the feed reaches temperatures of 200° to 350° F. for 45 to 60 seconds, no salmonellae were recovered.

It has been demonstrated that rendered products used in animal feeds can be heated sufficiently during processing to kill salmonellae without significant alteration of the nutritive value of the protein (Rasmussen, et al.); the primary problem lies in prevention of recontamination after processing and during transport to the distributor.

ARBOVIRUS ENCEPHALITIS - 1965
(Morbidity and Mortality Weekly Report)

A total of 134 confirmed or presumptive human cases of Arbovirus encephalitis has been notified in the United States through September 30, 1965. There have been 82 cases of Western equine encephalitis, six of Eastern equine encephalitis, 16 of St. Louis encephalitis, and 30 of California encephalitis. Of the 15 States affected, only two, Colorado and Texas, have had confirmed infection with more than one Arbovirus. Colorado has had the largest number of confirmed cases of Western equine and of St. Louis encephalitis of any one State.

Western Equine Encephalitis

A total of 82 laboratory confirmed and presumptive human cases of Western equine encephalitis has been reported through September 30 from seven central and western States. Human outbreaks of some size have occurred in Colorado which had 37 cases; Wyoming, 15 cases; North Dakota, 12 cases; and Texas with 14 cases.

Wyoming, North Dakota and Colorado have experienced more human Western equine encephalitis this year than in any year since encephalitis surveillance was established in 1958.

The ages of the patients varied from two months to 75 years. There was one fatality in Minnesota.

A concentration of human cases occurred in the Platt and Arkansas River Valleys of Wyoming and Colorado. In these areas there was heavy
flooding during the spring of 1965 which gave rise to unusually large mosquito populations. Western equine encephalitis virus was recovered from pools of *Culex tarsalis* early in July.

In the map, Figure 1, are shown the States reporting Western equine encephalitis in horses. The number of confirmed and presumptive human cases of Western equine encephalitis is also included.

*California Encephalitis*

Confirmed and presumptive human cases of California virus encephalitis have been notified from three midwestern States through September 30. Ohio reported 16 cases, with Wisconsin and Indiana each reporting seven. All the patients were children under 16 years of age, the youngest being three years old; all lived in rural areas or had been camping in forested areas prior to the onset of illness. There were no fatalities or sequelae.

Collections of mosquitoes and of sera from a wide variety of vertebrates have been made in the three States. To date there has been only one California virus isolation; it was obtained in Ohio from a pool of *Aedes triseriatus*.

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**Figure 1.** Western Equine Encephalitis in the United States through September 30, 1965.
St. Louis Encephalitis

There have been 16 confirmed and presumptive cases of St. Louis encephalitis, eight of which occurred in Colorado, six in Texas, and one each in Illinois and Pennsylvania. In Texas three of the cases occurred in Corpus Christi, while the other three appeared sporadically throughout the State. In Illinois there was one case in Enfield; St. Louis encephalitis virus has been isolated from a single pool of Culex pipiens collected in this area. There has also been one confirmed case reported from Collegeville, Pennsylvania, with the onset of illness during the first week in September.

It is of interest that there have not been any cases of St. Louis encephalitis reported from Houston, Texas, or Camden, New Jersey, both of which experienced major epidemics in 1964. McLeansboro, which is 12 miles west of Enfield in Illinois, was the site of a St. Louis encephalitis outbreak in 1964.

Eastern Equine Encephalitis

North Carolina, Georgia and Florida have reported one, two and three laboratory confirmed human cases of Eastern equine encephalitis respectively. Two fatalities occurred, both in Georgia. The latest confirmed human case this year was reported from Florida, the date of onset being July 24.

There is, however, continued evidence of Eastern Equine encephalitis virus activity in the eastern United States. Equine epizootics have been reported from Delaware, Florida, Georgia, Louisiana, Maryland, New Jersey, North Carolina, South Carolina, Virginia. Eastern equine encephalitis virus has been isolated from pools of Culiseta melanura collected in Florida, North Carolina, Virginia, Maryland and New Jersey; it has also been recovered from one pool of Anopheles crucians in New Jersey. Activity in birds has been demonstrated by the recovery of Eastern equine encephalitis virus from a sparrow in North Carolina and from four avian species in New Jersey, namely the house sparrow, woodthrust, Carolina Chickadee, and red-eyed vireo.

REFERENCES


THE PREVALENCE IN ILLINOIS SWINE OF NEUTRALIZING ANTIBODY TO A PORCINE ENTEROVIRUS, ECPO-6


INTRODUCTION

The presence of porcine enteroviruses in the swine population of Illinois has been suspected for some time; however, the existence of these agents was not established until the present study was conducted.

Reports on the pathogenicity of one porcine enterovirus, designated ECPO-6\(^1,2\) and observations on its possible involvement in swine respiratory disease\(^3\) emphasized the importance for more information on the prevalence of ECPO-6 in the state's swine population.

MATERIALS AND METHODS

**Serum Samples:** Forty-five serum samples from nine widely separated herds in Illinois were collected and examined for ECPO-6 neutralizing antibody. Samples from five animals in each herd were tested using the plaque reduction technique.\(^4\) Three of the herds examined were accredited specific pathogen free (SPF); the remaining herds were conventional as to the procurement and management of the animals. Five additional samples of serum from individual animals submitted to the diagnostic laboratory were also examined as were two samples of commercial hog cholera antisera. All samples were kept frozen at \(-20\) C until tested and 52 serum samples were tested in all.

**Plaque Reduction Tests:** Primary cultures of adult swine kidney cells were prepared in 4 oz. prescription bottles employing a medium of 0.5 percent lactalbumin hydrolysate supplemented with 10 percent sheep serum in Hanks Balanced Salt Solution (HBSS). When the monolayer cultures were confluent, a known concentration of ECPO-6 virus previously titrated by the plaque technique, 80 plaque forming units (PFU)/0.1 ml, was mixed with an equal volume of test serum diluted in HBSS.\(^4,5\) The virus-serum mixture was incubated for one-half hour at \(37\) C. The growth media was then removed from the cell cultures and 0.2 ml of each of the various virus-serum dilutions inoculated onto and distributed over the monolayer. Inoculated cultures were prepared in duplicate and incubated at \(37\) C for one-half hour to allow for virus adsorption, before overlayed with agar and incubated as described previously.\(^6\)

Controls consisted of cultures inoculated with (a) 0.2 ml of undiluted virus, (b) the diluted virus containing 80 PFU/0.1 ml mixed with an equal

From the University of Illinois College of Veterinary Medicine and Agricultural Experiment Station.

This study was supported in part by the Illinois Department of Agriculture Swine Disease Research program.

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volume of HBSS and (c) control cultures which received 0.2 ml of HBSS in place of the virus or virus-serum mixture.

The highest dilution of serum which resulted in an 80 percent or greater reduction in the number of observable plaques was considered the end point in determining the titer of the serum sample.

All serum samples were first examined for antibody using dilutions of 1:8 and 1:16. Samples with antibody activity were then selected at random from each herd, and tested at dilutions of 1:5, 1:50, 1:250, 1:500 and 1:1000.

RESULTS

The prevalence of antibody in the samples examined was considerably higher than expected, with 94 percent of the samples positive. Of positive serum samples selected for titration, Table IV, the majority had titers approaching 1:1000 or above.

The plaques formed by ECPO-6 were small and not readily observable until the fifth day and were counted for record on the sixth day.

The results of all serum samples tested using dilutions of 1:8 and 1:16 are presented in Tables I, II and III. Table IV presents the results of selected serum samples positive at 1:16 tested at the higher dilutions.

TABLE I

Three SPF Swine Herds Tested at Dilutions of 1:8 and 1:16 Against ECPO-6 Virus

<table>
<thead>
<tr>
<th>Herd</th>
<th>Animal</th>
<th>Serum dilutions</th>
<th>No. of plaques</th>
<th>Serum dilutions</th>
<th>No. of plaques</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A1</td>
<td>1:8</td>
<td>0</td>
<td>1:16</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>1:8</td>
<td>0</td>
<td>1:16</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>1:8</td>
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<td></td>
<td>A4</td>
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<td>0</td>
<td>1:16</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>B2</td>
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<td>0</td>
<td>1:16</td>
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</tr>
<tr>
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</tr>
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<td></td>
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<td>C</td>
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<tr>
<td></td>
<td>C5</td>
<td>1:8</td>
<td>0</td>
<td>1:16</td>
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</tbody>
</table>

Virus control—undiluted monolayer completely destroyed
Virus control—80 PFU/0.1 ml 78
Cell culture control 0

R. C. MEYER, ET AL.
PREVALENCE OF NEUTRALIZING ANTIBODY

TABLE II

Serum Samples from Six Conventional Herds Raised in Illinois Tested at Dilutions of 1:8 and 1:16 Against ECPO-6

<table>
<thead>
<tr>
<th>Herd</th>
<th>Animal</th>
<th>Serum dilutions</th>
<th>No. of plaques</th>
<th>Serum dilutions</th>
<th>No. of plaques</th>
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<td></td>
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<td></td>
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<td>1:16</td>
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<td>+</td>
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<tr>
<td></td>
<td>E3</td>
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<td>1:16</td>
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<td>+</td>
</tr>
<tr>
<td></td>
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<td>+</td>
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<tr>
<td></td>
<td>F2</td>
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<tr>
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<td>+</td>
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<td>+</td>
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<td>G</td>
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<td>1:8</td>
<td>0</td>
<td>1:16</td>
<td>0</td>
<td>+</td>
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</tbody>
</table>

ECPO-6 Virus Control—80 PFU/0.1 ml 74
Cell Culture Control 0

DISCUSSION

Since all the herds tested were positive and existed in widely separated counties (Figure 1), it is probable that the agent is disseminated throughout these counties and that the majority of the swine in the heavy production areas have been or will become infected. The status of the various herds whether they were SPF or conventional appears to bear little relationship to the prevalence of antibody. This indicates that ECPO-6 is highly transmissible and present procedures deemed adequate
TABLE III
Serum Samples from Individual Animals Submitted to the Diagnostic Laboratory and Commercial Hog Cholera Antisera Tested at 1:16

<table>
<thead>
<tr>
<th>Animal</th>
<th>Serum Dilutions</th>
<th>No. of plaques</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>1:16</td>
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<td>3</td>
<td>1:16</td>
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<td>+</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>5</td>
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<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

Commercial Sera I 1:16 0 +
Commercial Sera II 1:16 0 +

ECPO-6 Virus Control 80 PFU/0.1 ml 82
Cell Culture Control 0

TABLE IV
Selected Serum Samples Using Dilutions to 1:1000 Against ECPO-6

<table>
<thead>
<tr>
<th>Herd</th>
<th>Animal</th>
<th>Serum Dilutions</th>
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</tr>
<tr>
<td></td>
<td></td>
<td>No. of plaques</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>A4</td>
<td>0 0 45 72 1:250</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>B3</td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>C1</td>
<td>0 0 0 0 1:1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>85 plaques</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 plaques</td>
<td></td>
</tr>
</tbody>
</table>

Conventional

| D     | D4     | 0 0 0 46 1:500 |       |
| E     | E2     | 0 0 2 34 1:500 |       |
| F     | F4     | 0 0 0 5 1:1000 |       |
| G     | G3     | 0 0 0 9 1:1000 |       |
| H     | H1     | 0 0 24 81 1:250|       |

ECPO-6 Virus Control 85 plaques
Cell Culture Control 0 plaques

to maintain a herd free of specific pathogens do not suffice to prevent the entrance and spread of such an enterovirus. The occurrence of the virus in the SPF herds was a little surprising inasmuch as it was anticipated that if negative animals were to be found they would most likely exist in SPF swine.

The prevalence of antibody in the herds tested might appear high but are consistent with a similar study conducted in central Ohio where the serum was positive from 18 of 25 (72 percent) adult swine representing three separate herds.7

While the total number of samples tested is small compared to a swine population of eight million in Illinois, the sample is believed sufficient to substantiate the existence of ECPO-6 or a closely related agent
in Illinois herds. Since the agent is known to exist in Ohio it could very well be distributed throughout the swine producing areas of the Midwest.

A similar study conducted in western Missouri on another porcine enterovirus designated (MF) found 81 percent of the serum samples examined positive for antibody against this agent.\(^8\)

Porcine enterovirus have been reported to vary considerably in their capacity to evoke an antibody response.\(^9\) In this regard ECPO-6 invokes...
a good response\textsuperscript{2} and is evidenced by titers approaching or surpassing 1:1000 in the samples we examined.

The good antibody response may be explained by the fact that ECPO-6 is known to produce a viremia which often persists for the first few days of infection.\textsuperscript{1,2,3} Since the agent gains access to the blood stream, there is adequate stimulation of antibody producing centers. A second contributing factor may have been that most of the serum samples were from gilts and pregnant sows whose experience with the agent could have been relatively recent.

ECPO-6 is known to possess neurotropic properties\textsuperscript{1,2} but recent observations indicate that intranasal inoculations of the agent into young four to six week old colostrum deprived SPF pigs can also induce pneumonia.\textsuperscript{3} The role of coxsackie and ECHO virus in human respiratory disease is well documented.\textsuperscript{10} The porcine enteroviruses may very well play an unsuspected role in initiating respiratory disease in swine.

**SUMMARY**

Fifty-two serum samples representing accredited SPF and conventional herds as well as individual animals submitted to the diagnostic laboratory were examined for ECPO-6 neutralizing antibody.

Antibody was found in 94 percent of the samples examined. Classification of the herd as to SPF or conventional bore little relationship to the prevalence of antibody.

Since the serum samples positive for antibody were from widely separated herds within the State, it is suggested that the agent not only exists in Illinois swine but may be widespread.

**REFERENCES**

Transmissible gastroenteritis (TGE) of swine is a highly contagious and fatal disease of piglets, under two weeks of age, causing considerable economic loss in the Midwest of the United States. Research on this virus disease has been severely handicapped by the lack of an experimental host other than the pig. Recent reports have described the ability of TGE virus to produce a cytopathic effect (CPE) in cell cultures although the effect was usually faint or appeared only after a few blind passages.\textsuperscript{1,2,3}

This report describes additional use and value of cell culture methods for the isolation and study of TGE virus, with emphasis on the plaque technique. In addition, some of the common characteristics of the TGE virus isolates are described and compared with those of known strains.

**MATERIALS AND METHODS**

**Cell Culture**

Primary cultures of porcine kidney (PK) cells were grown in four ounce prescription bottles, similar to the methods previously described.\textsuperscript{4} Swine from one to three months of age were used as sources of kidneys. Growth medium consisted of Hanks' balanced salt solution, 0.5 percent lactalbumin hydrolysate, 10 percent heat-inactivated bovine serum and antibiotics. Maintenance medium used during the propagation of viruses was similar except it contained five percent bovine serum. Usually, a confluent monolayer of cells occurred after four to seven days at which time the bottles were then used for isolating or propagating viruses.

**Plaque Technique**

The procedure was similar to that which has been previously described.\textsuperscript{5} Only bottles showing a confluent sheet of healthy cells were used. Prior to adding virus, the fluid in the bottle was removed. The inoculum for each bottle was usually 0.2 ml. of the virus material, and an attempt was made to disperse the inoculum over the cellular monolayer by tilting the bottles back and forth several times. The inoculated bottles were incubated at 37°C for 90 minutes, during which time it was expected that most of the viral particles would be adsorbed.
onto the cells. About 12 to 15 ml. of the agar overlay medium (at 42 C.) was then added to each bottle. This medium consisted of: Hanks' balanced salt solution, 0.5 percent lactalbumin hydrolysate, five percent bovine serum, 0.9 percent Noble agar,* 0.0012 percent neutral red, 0.22 percent sodium bicarbonate, and antibiotics.

Neutralization Tests

The presence of neutralizing antibody for TGE virus in serum was detected by the plaque neutralization method. The test virus was suitably diluted so as to give approximately 50 plaque-forming-units (PFU) per 0.1 ml. Varying dilutions of the serum, under test, were made and were mixed in equal volumes with the diluted virus. The serum-virus mixtures were incubated at room temperature in the dark for 1 hour, followed by the inoculation of 0.2 ml. into each of at least two PK bottles. Agar overlay was then added after an adsorption period of 90 minutes. The dilution of serum which neutralized 50 percent of the PFU was considered the neutralizing titer (NT50). The calculation of the NT50 was by Kärber's method.6

Antibodies in colostrum and milk were detected in the same manner as described for serum except the fat was removed by centrifugation prior to testing. To facilitate the collection of colostrum and milk, the sows were injected intravenously with 30 units oxytocin.

All serum, colostrum, and milk samples were heated at 56 C. for 30 minutes prior to testing.

Cross neutralization tests were conducted with the cell-culture adapted SH, Purdue, and Station strains. The corresponding antisera were prepared as follows: The pig-passaged Purdue strain and the cell-culture adapted SH strain were each used to infect a germfree piglet. The pig-passaged Station strain was used to infect a conventional pig. Preinfection sera, diluted 1:4, of these three pigs did not neutralize the three cell-culture adapted strains. Immune sera were collected three weeks after exposure.

RESULTS AND DISCUSSION

Isolation of Viruses in Cell Cultures

Six field outbreaks of TGE were investigated and in each case the history and clinical signs were typical. Vomition, yellow watery diarrhea, dehydration, and a high mortality were observed in pigs under two weeks of age. In each of these herds, some of the sows and older swine showed diarrhea and inappetence.

Certain precautions were taken in order to obtain field specimens that would contain a high content of TGE virus and, also, tend to keep to a minimum the probability of such specimens containing enteroviruses. One pig, usually two to five days old, in the early stage of

*Difco Laboratories, Detroit 1, Michigan.
THE USE OF CELL CULTURES

the disease from each outbreak was sacrificed, and the small intestine was aseptically removed. In addition to these specimens from the field outbreaks, the small intestines of pigs infected with known strains of pig-passaged TGE virus; namely, the Purdue* and the NY II,** were used similarly for isolation purposes.

Ten percent suspensions of the small intestines were made using maintenance medium containing penicillin (250 units/ml.), streptomycin (250 µg./ml.), and mycostatin (250 units/ml.). The suspensions were centrifuged at 1500 g. for one hour at 4 C. and the supernatant fluids were removed and stored frozen until tested for the presence of a virus. Bottles of PK cell monolayers were emptied of media and inoculated with 0.1 to 0.2 ml. of the inoculum. After an adsorption period of two hours, either maintenance medium or agar overlay was added. During the first three passages, using maintenance medium, the fluid was harvested after four and eight days and pooled for a subsequent passage. CPE of a mild degree was observed in either the first or second passage from each of the 6 field specimens and the 2 known pig-passaged TGE strains. There was a rounding and detachment of cells from the monolayer, but in the early passages this would result in only a few, if any, bare spots in the cell sheet. However, after a few passages the CPE became more pronounced so that more than one-half of the cells were detached from the glass after four days. The degree and extent of CPE varied with different lots of PK cells, and the reason for this has not been determined; although some difference has been associated with the source of the bovine serum that is used in the growth and maintenance media.

In contrast to the slight and sometimes transient CPE observed with the use of the liquid medium, plaques usually occurred when agar overlay medium was used for the primary isolation from intestinal material, as shown in Fig. 1 (bottles C-1 and G-1). Different lots of PK cells varied in their sensitivity for showing plaques, as well as for showing CPE.

Work is underway to determine if serial passage in cell cultures can be used to reduce the virulence of the TGE virus. Several strains are being passed with this object in mind. So far, the Purdue strain has been passed 43 times but information on its attenuation is incomplete at this time. Obviously, there is interest in the possible use of an attenuated strain for immunizing swine.

Some Characteristics of Various Strains

The type of CPE and plaques produced by the six field strains, the Purdue strain, the NY II strain, and the Japanese SH strain† of TGE virus were compared and were found to be very similar. Plaques became more distinct and larger after a few passages in cell cultures. Figure 1 shows plaques of the SH and the Purdue strains. Plaques

*Obtained from Dr. E. O. Haelterman, Purdue University.
**Obtained from Dr. Ben Sheffey, Cornell University.
Figure 1. Plaques of four strains of TGE virus. Bottle C-1 was inoculated with 0.2 ml. of a 1:10 suspension of small intestine, Creston strain, 12-day-old plaques. Bottle G-1, with 0.2 ml. of a 1:1000 suspension of small intestine, Grubb strain, 12-day-old plaques. Bottles SH and Pur. (Purdue) represent six-day-old plaques of the cell-culture adapted strains.

usually became first evident after three days and gradually increased in size as long as the cell culture remained viable. The plaque technique was found to be more sensitive and reliable for the detection and titration of TGE virus than by the conventional method of observing CPE under liquid medium. The titers of the SH strain at the 29th passage and the Grubb strain (a field isolate) at the ninth passage were $10^6$ and $10^{4.1}$ PFU/ml., respectively.

Typical clinical signs of TGE were observed in young piglets following the oral administration of each of the cell-culture adapted strains from the six field cases, the SH strain, and the cell-culture adapted Purdue strain. The Grubb strain of the ninth passage was used to infect a 17-day-old "germfree" pig, using germfree techniques as previously reported. Vomition and a yellow watery stool was first noticed 36 hours after infection, and the animal was sacrificed 24 hours later. Rectal swabs were examined for fungi, and bacteria, including Mycoplasma, but none were recovered. There was a marked shortening of the villi of the jejunum, which, according to Haelterman and Hooper, is a characteristic finding for TGE. This shortening of the villi has also been observed in piglets which were infected with the Grubb strain and which were nursing the sow.

Four of the field strains (Grubb, Station, Miller, Luersman), the SH, and the Purdue strains were exposed to 25 percent diethyl ether for two hours at room temperature. Each was found labile as there was a reduction of at least $10^{2.5}$ PFU/0.1 ml. for each strain. As controls, ECPO-5 virus, a porcine enterovirus, and infectious bovine rhinotracheitis (IBR) virus, a member of the herpesvirus group, were tested and found resistant and sensitive, respectively. These findings that the TGE virus is labile to ether are in agreement with other
and indicate that this virus should not be classified as an enterovirus. The SH and Grubb strains were tested for their ability to produce plaques in the presence of agar medium containing 50 µg. of five iodo deoxyuridine (IDU)/ml. This chemical inhibits the growth of DNA viruses in cell cultures. There was no significant reduction in the number or the size of plaques with either strain. As controls, ECPO-5 virus was likewise not inhibited, but with IBR and pseudorabies viruses there were reductions in the number of plaques by at least 99 percent. The latter two viruses are of the DNA type. These findings suggest that the TGE virus is of RNA type.

The SH and Grubb strains were stable when exposed at pH three for 30 minutes, using Tris (hydroxymethyl) aminomethane-HCl buffer as the suspending medium. No hemadsorption of guinea pig red blood cells occurred in cell cultures infected with either of these strains. The Grubb strain, suspended in maintenance medium, was exposed to 50 C. for 30 minutes, resulting in a drop in titer from $10^{4}5$ to $10^{2.6}$ PFU/ml., amounting to an inactivation of 98.6 percent of the PFU.

The viruses studied in this report resemble those of the myxovirus or arbovirus groups in respect to RNA type and lability to ether and temperature, but differ in respect to stability at a pH of three. However, there may be some question as to whether lability at a pH of three is a satisfactory criterion for classifying myxoviruses. It is probably not an arbovirus as there is no evidence that it multiplies in or is transmitted by arthropod vectors. Ristic et al. recently described the electron microscopic appearance of a virus which was obtained and purified directly from the small intestine of pigs showing clinical signs of TGE; and they stated that its structural characteristics were similar to those of the herpesvirus group. According to our work the TGE virus would be considered an RNA virus and, thus, it should not belong in the herpesvirus group. Obviously, more work is necessary for the proper classification of this virus.

Serologic Studies

Serologic studies on TGE were conducted in four infected herds. These herds represented separate outbreaks, as each was at least 25 miles from the other. In all, nine sows from these four herds were bled during the acute and the convalescent (two to three weeks later) phases of the disease. Each sow had shown diarrhea or inappetance and had nursed sick pigs. The plaque neutralization test using the SH strain was employed in testing these samples. All of the acute serum samples were negative at a dilution of 1:4, while all of the convalescent serum samples were positive at dilution of 1:32 or above. Several of these samples were also tested with the Grubb and Miller strains (field isolates) and the same results were obtained. These results further indicate a causal relationship of these strains of viruses with TGE. More detailed serologic studies were conducted on serum samples of
two of these sows, as indicated in Table I. The results showed similar antibody titers with the SH, Purdue, and Station strains.

The results of the cross neutralization tests involving the SH, Purdue, and Station strains are shown in Table I. Similar titers were obtained with the homologous and heterologous strains with all the antisera indicating close antigenic similarity between one Japanese and two American strains of TGE virus. A 1:64 dilution of the Purdue strain antisera neutralized more than 50 percent of the plaques of each of the field isolates and the NY II strain.

**TABLE I**

Cross Neutralization of Strains of TGE Virus

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<thead>
<tr>
<th>Sera*</th>
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<td></td>
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<td>Purdue</td>
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<td>3102</td>
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<td>Convalescent sera</td>
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<td>7125</td>
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<tr>
<td>Miller No. 1</td>
<td>181</td>
</tr>
</tbody>
</table>

*See text for sources of sera.

**NT50 = Reciprocal of the serum dilution which showed 50 percent neutralization of the PFU.

In some preliminary findings, TGE antibodies, in rather high titer, were detected in the colostrum and milk samples of two sows artificially infected with the Miller strain (20th cell-culture passage), as shown in Table II. The plaque neutralization test is a sensitive and reliable method of antibody assay for TGE and should prove most useful as a means of evaluating the ability of immune sows to transmit protection to their nursing pigs.

**SUMMARY**

Cell-culture methods were used to isolate a CPE-producing virus from each of six outbreaks of TGE. Viruses of the same characteristics were isolated from known strains of TGE virus that had been maintained only in pigs; namely, the Purdue and NY II strains. The cell-culture adapted Japanese SH strain was compared with the American strains and no significant difference was detected. Cross neutralization
THE USE OF CELL CULTURES

TABLE II
Antibody Titers in the Serum, Colostrum and Milk of Experimentally Infected Sows*

<table>
<thead>
<tr>
<th>Sow #</th>
<th>Sample</th>
<th>Days Post-infection</th>
<th>Days Post-farrowing</th>
<th>Antibody titer**</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>Serum</td>
<td>0</td>
<td></td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>20</td>
<td>0</td>
<td>587</td>
</tr>
<tr>
<td></td>
<td>Colostrum</td>
<td>21</td>
<td>0</td>
<td>&gt; 256</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>23</td>
<td>2</td>
<td>&gt; 64</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>44</td>
<td>23</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>588</td>
<td>Serum</td>
<td>0</td>
<td>0</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>20</td>
<td></td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td>Colostrum</td>
<td>33</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>35</td>
<td>2</td>
<td>&gt; 64</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>44</td>
<td>11</td>
<td>&gt; 64</td>
</tr>
</tbody>
</table>

*Both sows orally infected with the Miller strain of TGE virus (20th cell-culture passage).

**Reciprocal of serum dilution neutralizing 50 percent of the PFU. Miller strain was used in the neutralization test.

tests on several of these strains revealed no significant antigenic differences.

The plaque technique was found to be useful for isolating and titrating this virus. Using the plaque neutralization test, antibodies were detected and titrated in the serum of naturally and artificially infected swine. Antibodies in appreciable titer were detected in the colostrum and milk of sows which had been infected with TGE virus.

Cell-culture methods will be of great value for a detailed study of TGE.

ACKNOWLEDGMENTS

The authors wish to thank Drs. A. Trapp, C. Vonderwell, L. Schultzman, A. Ebert, F. Haberman, and P. Klinefelter for their assistance in collecting field specimens, and to Mrs. Linda Thomas for technical assistance.

REFERENCES


CHARACTERIZATION OF TRANSMISSIBLE GASTROENTERITIS VIRUS

Ben E. Sheffy*

Transmissible gastroenteritis (TGE) has been considered one of the most serious diseases of swine. Finding a viral etiology helped in understanding the infectious nature of TGE but this made possible little control except that induced by infecting sows with virulent virus and consequent protection of their pigs through ingestion of colostrum. Further development of knowledge depended on tissue culture. First attempts at cultivation failed to yield a cytopathogenic strain; continued efforts, however, lead to success. Characterization of the virus thus became possible and results accumulated thus far are reported here.

MATERIALS AND METHODS

Cultivation of Virus.—A strain of TGE virus, designated Shizuoka (SH), that had been transferred for 25 passages in pig kidney cell (PKC) cultures was received from T. Kumagai, National Institute of Animal Health, Tokyo, Japan by courtesy of E. O. Haeltman, Purdue University, Lafayette, Indiana. Upon receipt, it was transferred twice in rolled PKC cultures with medium Parker 199 without serum. Then, two additional transfers were made in rolled cultures at terminal dilutions using medium Parker 199 with five percent bovine serum. Cytopathic effects observed were foci of rounded cells that developed 36 to 48 hours after inoculation. These cells became separated from the glass leaving clear plaques surrounded by degenerating cells.

Primary pig kidney cell cultures were initiated with Earle's solution that contained 0.5 percent lactalbumin hydrolysate, antibiotics and 10 percent bovine serum. After cell monolayers had become confluent, various combinations of Earle's medium, Eagle's medium and P199, with and without lactalbumin hydrolysate, glutamine and either with lamb or with bovine serum were tested for effect on SH-TGE virus multiplication (Table I). For all culture media, an antibiotic mixture was used that consisted of 50,000 units of penicillin, 100,000 units of mycostatin and 100 mg. of streptomycin per liter. Parker 199 with five percent bovine serum showed the highest titer and was used in all of the studies reported here except those in which halogenated uridine compounds were tested. For these studies, Earle's medium replaced P199.

In order to determine incubation time for optimum virus production, stationary PKC cultures were washed twice with warm phosphate buffered saline solution (PBS) and each culture was inoculated with 0.1 ml. of

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Effect of Various Tissue Culture Media on Growth of SH-TGE Virus

<table>
<thead>
<tr>
<th>Number</th>
<th>Medium*</th>
<th>Log10 TCID50/0.1 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Earle's 4% lamb serum</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>P199 5% lamb serum</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>P199 5% bovine serum</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>Eagle's 5% bovine serum</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>Earle's 5% bovine serum</td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>P199 5% bovine serum</td>
<td>4.5</td>
</tr>
<tr>
<td>7</td>
<td>Earle's 5% bovine serum</td>
<td>4.2</td>
</tr>
<tr>
<td>8</td>
<td>Eagle's 5% bovine serum</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*Media 1, 3 and 5 contained lactalbumin hydrolysate (5.0 gms. per liter); medium 4 contained L-Glutamine (0.29 gms. per liter); and all media contained 50,000 units penicillin, 100,000 units mycostatin and 100 mg. streptomycin per liter.

SH-TGE virus that showed a titer of 4.5 logs per 0.1 ml. After one hour at 36°C, excess fluids were removed and the cultures again were washed twice with warm PBS. These fluids and washings were pooled and, when titrated for unadsorbed virus, more than 90 percent was found to be adsorbed. Then P199 medium with five percent bovine serum was added and incubation was continued. Two cultures were removed at three hour intervals for the first 12 hours and thereafter at 12 hour intervals for the next 120 hours. Supernatant fluids from tubes at each time interval were pooled and were freed of cellular debris by centrifugation at 1500 rpm for five minutes. Each pool was mixed with equal parts SPGA1 buffer and held at -70°C. until tested for virus. The cells that remained attached to the glass were washed twice with warm PBS, then 1.5 ml. of P199 plus five percent serum was added to each tube. After tubes were frozen and thawed several times, fluids were pooled, centrifuged at 1500 rpm for five minutes, mixed with equal parts SPGA buffer and stored at -70°C. until tested for virus. Virus concentrations in fluid and the cell associated virus have been plotted in Figure 1.

Figure 1 indicated that virus in the fluid phase should be harvested two to three days after inoculation. All subsequent virus transfers, therefore, have been made in stationary PKC cultures with P199 plus five percent bovine serum. After incubation for three days at 36°C., fluid was removed and frozen and thawed. Then cell debris was removed by low

### SPGA Formula

<table>
<thead>
<tr>
<th>Item</th>
<th>gm/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>200.0</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.45</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.35</td>
</tr>
<tr>
<td>Monopotassium L. glutamate</td>
<td>0.956</td>
</tr>
<tr>
<td>Porcine albumin, fraction (Armour)</td>
<td>10.0</td>
</tr>
</tbody>
</table>
CHARACTERIZATION OF TGE VIRUS

GROWTH CURVE OF SH TGE VIRUS

Figure 1

speed centrifugation, supernatant was mixed with equal portions of SPGA buffer and placed at -70°C. for further use.

Temperature stability. — A suspension of tissue cultured SH-TGE virus that had been mixed with equal parts of SPGA buffer (pH 7.2) was distributed into glass ampules in one ml. amounts. After sealing, they were placed at the following temperatures: -30°C., 4°C., 22°C., 37°C., 45°C. and 56°C. At various intervals of time, an ampule was removed and immediately placed in an ice bath. Then tenfold dilutions were made and each of three tubes of PKC cultures was inoculated with 0.1 ml. of a dilution. The cultures were observed for six days and cytopathic changes were recorded (Figure 2).

Light sensitivity. — As for temperature studies, sealed ampules that contained one ml. of a suspension of TGE virus at pH 7.2 were held at room temperature, some in darkness while others were exposed to daylight and light from fluorescent lamps in the laboratory. At varying intervals of time, virus content of an ampule was determined. For
ultraviolet light sensitivity, suspensions of SH-TGE virus were placed in petri dishes to a depth of one mm. and exposed to ultraviolet light from an eight watt lamp at a distance of 18 centimeters. At specified time intervals, aliquots were removed and titrated for virus.

Ultrafiltration. — For particle size estimation, a suspension of virus was filtered through membrane filters (Millipore Filter Corp.) of 50–100, of 150–200, of 250–400 and of 400–800 pore diameters in the manner recommended by the manufacturer. Unfiltered virus suspension and each filtrate were titrated for virus content (Table II).

Ether and chloroform sensitivity. — Absolute diethyl ether (Mallinckrodt) was added to a suspension of SH-TGE virus in a final concentration of 20 percent and allowed to stand for 18 hours at 4°C. Then the mixture was centrifuged at 2000 rpm for 20 minutes and the ether layer removed. Controls consisted of infectious canine hepatitis virus similarly treated and of SH-TGE virus suspension in which 20 percent PBS replaced the ether. Analytical reagent grade chloroform (Mallinckrodt) was added to a suspension of SH-TGE virus in a final concentration of five percent. After continuous shaking for 10 minutes at room temperature, the mixture was centrifuged at 1500 rpm for five minutes, supernatant was removed and was titrated for virus. Infectious canine hepatitis virus and untreated SH-TGE virus was included.

pH stability. — A suspension of SH-TGE virus was dialyzed against frequent changes of 0.005 M. phosphate buffered saline (pH 7.0) at 4°C. for three days. Then, one volume of dialyzed virus was mixed with two volumes of isotonic veronal-acetate buffer at pH values of three, five, seven and nine. Each mixture was placed at temperatures of 37°C. and at 20°C. At the time incubation began and at 15 minute intervals, aliquots of virus from each test were placed in an ice bath, pH adjusted to 7.2 by addition of isotonic veronal-acetate buffer and titrated for virus (Table III).

Cationic stability. — A two M. solution of MgCl₂ and of CaCl₂ was prepared in distilled water and sterilized by autoclaving. Each cationic solution and distilled water was mixed with an equal volume of undiluted SH-TGE virus. The mixtures were placed in a 50°C. water bath. At the time the test began, 30 minutes and 60 minutes later, each mixture was titrated for virus (Table IV).

Trypsin sensitivity. — SH-TGE virus was dialyzed against frequent changes of 0.02 M. phosphate buffered saline (pH 7.8) at 4°C. for three days. Then an equal portion of dialyzed virus was mixed with trypsin solution that had been prepared by dissolving trypsin (Difco) in 0.02 M. phosphate buffered saline (pH 7.8) at a concentration of one mg. per ml. and sterilized by filtration. In addition, virus was mixed with equal portions of buffer to serve as controls. Portions of the virus–trypsin and virus–buffer mixtures were assayed for infective virus prior to and one hour after incubation in a water bath at 37°C.

Nucleic acid type. — Two halogenated uridine compounds, 5 fluoro - 2 deoxyuridine (FUDR) and 5 iodo - 2 deoxyuridine (IUDR) were tested for inhibition of multiplication of SH-TGE virus as described by Hamparian
et al.\textsuperscript{11} and Lam and Atherton.\textsuperscript{12} Cultures of a known DNA virus, pseudorabies, and a known RNA virus, bovine virus diarrhea, were similarly treated.

\textit{Serological studies.—} Comparisons were made between SH-TGE virus and other strains and with other animal viruses including hog cholera, porcine enterovirus (T-80) and pseudorabies of swine; canine distemper, infectious canine hepatitis, and canine herpesvirus of dogs; and infectious bovine rhinotracheitis, bovine adenovirus and parainfluenza virus of cattle.

For preparation of serums, SPF Beagle dogs were divided into three groups of two each. In one group, each dog was fed 20 ml. of a 10 percent suspension of ileum from a pig infected with virulent NY II TGE virus. In another group, each dog was fed 20 ml. of tissue culture SH-TGE virus that had been transferred 31 passages and in the remaining group, each dog was inoculated intramuscularly with one ml. of tissue culture virus that had been transferred for 32 passages. Serums were obtained at the time of inoculation and again 30 days later. The Newark TGE serums were obtained from individual sows at the Newark State School several months after recovery from a natural occurrence of TGE. The Iowa serums were kindly supplied by Dr. J. Welter, Diamond Laboratories, Des Moines, Iowa as random samples from vaccinated sows. NY II TGE and SH-TGE serums were prepared in SPF pigs. The porcine enterovirus (T-80) immune serum was obtained by the courtesy of A. O. Betts, University of London, London, England. Antiserums for other viruses had been prepared in our laboratories and all had been shown to neutralize the respective viruses against which they had been prepared.

All serums were tested against SH-TGE virus by neutralization. In the test, serums were heated for 30 minutes at 56°C. and tenfold dilutions were prepared with P199 medium. Each dilution was mixed with equal parts of a virus suspension diluted to contain approximately 100 TCID\textsubscript{50}/0.1 ml. and held at 4°C. for two hours. Then each of three cell cultures was inoculated with 0.2 ml. of a serum-virus mixture and incubated at 36°C. Observations were made daily and final results were recorded six days afterwards. Tests were repeated at least twice (Table V).

\textit{Hemagglutination.—} TGE virus was tested for ability to hemagglutinate bovine, porcine and human type 0 erythrocytes. Twofold dilutions of virus that ranged from one to two through 512 were prepared. Equal portions of each virus dilution and a .25 percent suspension of washed erythrocytes were mixed and held at 4°C., 22°C. and 37°C. Results were recorded four hours and 18 hours afterwards.

\textit{Immunological studies.—} In a study to determine immunological response of young pigs and sows to TGE virus, tissue culture SH-TGE virus and NY II TGE virus that came from infected pigs were used. In one test, six littermate pigs that were two weeks of age were divided into two groups and each group was placed in a separate isolation unit. In one group, two pigs each were inoculated intramuscularly with two ml. of tissue culture fluids that contained SH-TGE virus after 47 passages. The remaining pig in the group was placed in contact as an uninoculated
control. In the other group, each of two pigs was fed five ml. of the SH-TGE virus that had transferred for 47 passages while the remaining pig was left in contact. Temperatures were recorded daily and clinical signs of illness were noted. Then three weeks later, each pig in both groups was inoculated intravenously with 10 ml. of SH-TGE virus at the 47th passage. At the time of initial inoculation, at the time of the second inoculation and one week later, all pigs were bled and serum tested for TGE antibody.

In another test, nine littermate pigs were divided into three groups and each group was placed in a separate isolation unit. In one group, each pig was fed five ml. of a 10 percent suspension of ileum from a pig infected with NY II TGE virus. In the second group, each pig was fed five ml. of SH-TGE virus that had been transferred for 32 passages while in the third group each pig was inoculated intramuscularly with two ml. of the tissue cultured virus. Serums were obtained from all pigs at the time of inoculation and again 30 days later. They were tested for neutralizing antibody.

In a test of response to TGE and ability to protect pigs by colostrum transfer, each of three sows was inoculated intramuscularly with two ml. tissue cultured SH-TGE virus that had been transferred 52 passages. One of the sows was inoculated two weeks before parturition while the other two were given virus four weeks before parturition. Sows were observed daily for signs of illness. All sows farrowed on schedule. Then when pigs were four days of age, each pig was fed two ml. of a 10 percent suspension of ileum that contained virulent NY II TGE virus. All pigs were left with the sows except six which were removed from two sows and hand-reared in an isolation unit. Pigs were observed daily (Table VI). Serums were obtained from each sow at the time of inoculation, at parturition and at the time the pigs were weaned. Also, samples of colostrum were obtained. Serums were procured from each pig 24 hours after parturition, at the time of infection with TGE virus and at weaning time. All serums and colostrum samples were assayed for TGE antibody (Table VII).

RESULTS

Characterization of virus.—Virus infectivity was destroyed at 56°C.; more than two logs of infectivity was lost after 15 minutes incubation and less than one log remained after 45 minutes (Figure 2). After incubation at 37°C. for 24 hours, 2.5 logs of virus infectivity remained. At room temperature, a three log loss in infectivity was found after 11 days. At 4°C., there was initial drop of approximately one log during the first 24 hours, thereafter infectivity remained essentially unchanged for 16 days, then there was a gradual but constant loss. Virus was found less stable at -30°C., than was found at 4°C. No significant loss of infectivity was recorded after 90 days storage at -70°C.

Although exposure of TGE virus to the daylight and fluorescent light in the laboratory decreased infectivity, differences were not significant.
Findings showed that, after eight days exposure to light, virus titrated log 1.5 in contrast to log 1.8 when held in the dark. Exposure of virus to ultraviolet light caused a 95 percent reduction in virus after five minutes exposure at room temperature and inactivation after 12 minutes.

SH-TGE virus readily passed millipore filters whose average pore diameters were 150-210 millimicrons and greater. Small quantities of virus passed the 50-100 μm filters (Table II). Using the filter factor suggested by Black,13 the hydrated particle size of TGE virus was estimated to be 81 μm.

TABLE II
Determination of Size of TGE by Ultrafiltration through Membrane Filters

<table>
<thead>
<tr>
<th>Filter Size*</th>
<th>Virus Titer after Filtration**</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-100</td>
<td>1.2</td>
</tr>
<tr>
<td>150-210</td>
<td>3.1</td>
</tr>
<tr>
<td>250-400</td>
<td>3.8</td>
</tr>
<tr>
<td>400-800</td>
<td>4.1</td>
</tr>
<tr>
<td>Control (Pre-filtration)</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*Average pore diameter in millimicrons.
**Titer expressed as TCID₅₀/0.1 ml inoculum.
Virus infectivity was destroyed by chloroform in a final concentration of 0.5 percent within 10 minutes. Treatment with diethyl ether at 4°C. inactivated virus in 18 hours. Virus suspended in PBS and handled in the same manner showed no loss of titer. Infectious canine hepatitis virus similarly treated was unaffected.

### TABLE III
Thermal Stability of TGE Virus in Buffer Solutions at Various pH Values

<table>
<thead>
<tr>
<th>pH</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>0 15m. 30m. 45m. 60m. 75m. 90m.</td>
</tr>
<tr>
<td>3</td>
<td>3.2 2.2 2.2 1.5 1.8 1.5 1.5</td>
</tr>
<tr>
<td>5</td>
<td>2.5 3.1 3.5 2.8 2.8 2.2 2.5</td>
</tr>
<tr>
<td>7</td>
<td>3.0 2.8 2.5 2.5 2.8 2.5 2.2</td>
</tr>
<tr>
<td>9</td>
<td>2.8 2.8 2.5 3.1 2.5 2.5 2.5</td>
</tr>
</tbody>
</table>

| 20±2°C | 0 15m. 30m. 45m. 60m. 75m. 90m. |
| 3  | 3.2 2.2 2.5 1.8 1.8 1.5 1.5 |
| 5  | 2.5 2.5 2.8 2.5 2.8 2.8 2.5 |
| 7  | 3.0 2.8 2.5 3.1 3.5 3.1 2.8 |
| 9  | 2.8 3.1 3.5 3.1 3.5 3.0 2.8 |

At pH 3, approximately two logs of virus was lost in 45 minutes at 37°C. and at room temperature. No further loss was recorded after 90 minutes. At pH values of five, seven and nine, no inactivation was detected at 37°C. or room temperatures after 90 minutes (Table III). Concentrations of 1 M. MgCl₂ and of 1 M. of CaCl₂ enhanced thermal inactivation of TGE virus when held at 50°C. for 60 minutes, (Table IV) when compared to inactivation of the virus in distilled water. TGE virus was resistant to trypsin at pH 7.8 for one hour at 37°C.

### TABLE IV
Effect of 1 M. Concentration of Cations on Stability of SH-TGE Virus at 50°C.

<table>
<thead>
<tr>
<th>Item</th>
<th>Virus Titer TCID₅₀/0.1 ml. after Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Min.</td>
</tr>
<tr>
<td>D.W.</td>
<td>3.8</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>3.3</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>3.1</td>
</tr>
</tbody>
</table>

IUDR and FUDR did not inhibit replication of SH-TGE virus when incorporated into the tissue culture medium at 0.0001 M. and at 0.00001 M. Similarly no inhibition was observed on the multiplication of BVD virus. Pseudorabies virus, a DNA type, was inhibited in the presence of 0.0001 M. IUDR. SH-TGE appeared to be a RNA type virus.

**Serological findings.**—All serums from dogs and from pigs prepared with various strains of TGE virus neutralized SH-TGE virus. None of the
10 antiserums prepared against viruses other than TGE virus neutralized (Table V). No hemagglutination of bovine, porcine or human type 0 erythrocytes was observed when SH-TGE antigen was tested at 4°C., 22°C. and 37°C., after four and after 18 hours of incubation.

**TABLE V**  
Relationship of SH-TGE Virus to Other Strains and to Other Viruses

<table>
<thead>
<tr>
<th>Serums</th>
<th>Results by Neutralization Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGE Virus</td>
<td></td>
</tr>
<tr>
<td>New York II</td>
<td>+</td>
</tr>
<tr>
<td>Newark</td>
<td>+</td>
</tr>
<tr>
<td>Iowa</td>
<td>+</td>
</tr>
<tr>
<td>SH-TGE</td>
<td>+</td>
</tr>
<tr>
<td>Hog Cholera Virus</td>
<td></td>
</tr>
<tr>
<td>Strain A</td>
<td>-</td>
</tr>
<tr>
<td>Strain Ames</td>
<td>-</td>
</tr>
<tr>
<td>PAV-1</td>
<td>-</td>
</tr>
<tr>
<td>Pseudorabies</td>
<td>-</td>
</tr>
<tr>
<td>Porcine Enterovirus T-80</td>
<td>-</td>
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<tr>
<td>Snyder Hill Canine Distemper</td>
<td>-</td>
</tr>
<tr>
<td>Infectious Canine Hepatitis</td>
<td>-</td>
</tr>
<tr>
<td>Canine Herpes Virus</td>
<td>-</td>
</tr>
<tr>
<td>Bovine Virus Diarrhea</td>
<td>-</td>
</tr>
<tr>
<td>Infectious Bovine Rhinotracheitis</td>
<td>-</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>-</td>
</tr>
<tr>
<td>Bovine Adenovirus 10</td>
<td>-</td>
</tr>
</tbody>
</table>

*Immunological findings.*—Pigs that were two weeks of age showed a transitory softening of feces within two to three days after inoculation either orally or intramuscularly with SH-TGE virus that had been transferred 47 passages. Temperatures remained normal. Neutralizing antibodies developed in the two pigs that were inoculated intramuscularly. Those fed or exposed by contact produced no antibody. Then seven days after another inoculation intravenously, pigs previously infected intramuscularly showed antibody titers greater than the contact pigs or those previously infected by the oral route. These data would indicate that all test pigs, except those given SH-TGE intramuscularly, had not been infected enough to produce antibody.

When six weeks old SPF pigs were fed virulent NY II TGE virus, slight temperature elevations and a diarrhea were found on the following day that lasted for three days. No clinical signs were seen in any pig infected with the 32nd passage of SH-TGE virus. All pigs infected with NY II TGE virus and with SH-TGE inoculated intramuscularly developed neutralizing antibodies. Only one of three pigs given SH-TGE virus orally showed antibody. Antibody titer produced with attenuated SH-TGE virus was lower than that with virulent NY II TGE.

No clinical signs of illness were observed when pregnant sows were
inoculated intramuscularly with SH-TGE virus that had been transferred 52 passages. Pigs appeared normal when farrowed. Each sow showed neutralizing antibody at time of farrowing. Measurable antibody was present in colostrum and four day mammary secretion in both sows infected four weeks prepartum. No antibody, however, could be measured either in the mammary secretion or the serum of pigs on the first and fourth day after nursing the sow that had been infected two weeks before parturition. When her pigs were given virulent TGE virus, all were protected. In the other sows, four of five and six of 10 pigs were protected (Table VI). All pigs showed vomiting and diarrhea that began 24 hours after feeding virulent virus, which ceased after two to three days except those that died. No clinical signs of illness were observed in the sows after exposure of pigs to virulent virus. Antibody titers of the sows at the time of weaning increased after exposure to virulent virus. All surviving pigs developed TGE antibody titers (Table VII).

TABLE VI
Survival of Pigs that Came from Sows Inoculated with SH-TGE Virus Following Exposure to Virulent NY II TGE Virus

<table>
<thead>
<tr>
<th>Litter</th>
<th>Not Nursed</th>
<th>Nursed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow 1</td>
<td>0/2</td>
<td>4/5</td>
</tr>
<tr>
<td>Sow 2</td>
<td>0/4</td>
<td>10/10</td>
</tr>
<tr>
<td>Sow 3</td>
<td>None</td>
<td>6/10</td>
</tr>
</tbody>
</table>

* Numerator - number pigs that survived; denominator - number of pigs tested.

TABLE VII
Serological Findings on Pigs and Their Dams that Had Been Inoculated with SH-TGE Virus and when Pigs were Four Days of Age Exposed to Virulent NY II TGE Virus

<table>
<thead>
<tr>
<th>Sow</th>
<th>Vaccination Time Weeks</th>
<th>TGE Antibody Titer Log₁₀/0.1 ml.</th>
<th>Pigs (Days of Age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
<td>Colostrum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 day vac.</td>
<td>4 day part.</td>
</tr>
<tr>
<td>Prepartum</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Vac. - vaccination; part. - parturition.
DISCUSSION

In confirmation of workers who isolated the SH-TGE strain in Japan,\textsuperscript{4} this strain was found to produce cytopathic effects in tissue cultured pig kidney cells within 36 to 48 hours. After inoculation with SH-TGE, serums from pigs and dogs neutralized virus as did serums from three American strains, two from New York and one from Iowa. Serums that neutralized a variety of other animal viruses failed to neutralize SH-TGE virus. A specific neutralizable tissue cultured virus thus made further work possible.

Multiplication and release of virus from pig kidney cell cultures was rapid; maximal infective virus was produced after 36-48 hours incubation at 36°C. This correlated with the rapid onset of clinical signs seen in newborn pigs infected with TGE virus. Virus infectivity declined when cultures were incubated for 144 hours, suggesting heat lability and, in agreement with a previous report,\textsuperscript{5} this was confirmed by stability tests at 37°C., 45°C. and 56°C. At room temperature however, TGE virus was more stable than previously reported.\textsuperscript{5} Also, photosensitivity of TGE virus under light conditions in our laboratory and effect of ultraviolet light was not different from that of hog cholera and bovine virus diarrhea virus similarly exposed.

As determined by ultrafiltration, size of SH-TGE virus was found to be approximately 81 \(\mu\text{m}\), other workers have reported a particle size of 80 \(\mu\text{m}\).\textsuperscript{5} Electron microscopy studies showed the virus size to be 98 \(\mu\text{m}\).\textsuperscript{14} Other physical and chemical studies showed TGE virus was inactivated by lipid solvents, unstable in 1 M. CaCl\(_2\) and 1 M. MgCl\(_2\) solutions, unstable at pH 3.0, not affected by trypsin and not inhibited by IUDR and FUDR. These characteristics suggested that TGE virus belongs in the Myxovirus group.

Pigs that came from sows recovered from virulent TGE were protected against virulent TGE virus as long as they nursed their dams.\textsuperscript{15} Of 25 pigs that nursed sows previously inoculated with attenuated SH-TGE, 20 survived although they showed diarrhea, while six littermate pigs that did not nurse died. Sows that were inoculated with SH-TGE virus did not develop antibody titers as high as sows which recovered from virulent TGE. Consequently, amount of protection transferred through colostrum was not as great. Amount of virus used to test protection of pigs was that contained in a 10 percent suspension of ileum from pigs infected with virulent virus (approximately 100,000 ID\(_{50}\)) and this amount may have represented an unfair test of protection when compared with exposure under field conditions. Contact exposure or lesser amounts of virus may have yielded a different result. It is of interest to note that those pigs which survived grew normally; apparently no virus persisted to cause the stunting observed by others.\textsuperscript{16}

If confirmed by further test, failure of attenuated SH-TGE virus to transmit from inoculated pigs to others placed in contact and ability to induce protection in pigs by nursing inoculated sows, tissue cultured live TGE virus could be a means of biological control through vaccination of
sows. Whether SH-TGE or another with similar characteristics was used would be unimportant because all strains of TGE studied thus far have been found to be antigenically similar.

SUMMARY

When cultivated in stationary PKC cultures in medium P199 with five percent bovine serums at 36°C., the SH strain of TGE virus showed maximum growth of virus within two to three days. Physical and chemical studies of tissue cultured virus indicated it possessed characteristics common to the Myxovirus group.

Cytopathic changes induced by SH-TGE virus in tissue culture consisted of foci of rounded cells that separated from the glass leaving clear plaques surrounded by degenerating cells. When tissue cultured virus was mixed with serums of pigs that recovered from TGE, virus was neutralized. A serum neutralization test was developed.

Virus that had been transferred in tissue culture for 45 passages caused no signs of illness when fed or inoculated intramuscularly into young pigs. Neutralizing antibodies were produced when virus was inoculated intramuscularly but not when given orally. Three pregnant sows that had been inoculated intramuscularly with virus after 52 passages protected 20 or 25 nursing pigs when they were fed virulent virus at four days of age while six littermate pigs that did not nurse died after virulent virus was fed. Inoculation of sows with SH-TGE virus thus offers a means of protecting their pigs against TGE.

ACKNOWLEDGEMENTS

The excellent technical assistance of Mrs. Marion I. Bowman and Mrs. Dorothy Mazzocchi is gratefully acknowledged.

REFERENCES

CHARACTERIZATION OF TGE VIRUS

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DYSES OF SWINE


The broad scope of the Committee results in attention being given to many important problems confronting the swine industry. Within these large entities are facets which have continued to merit discussion among regulatory officials and research scientists. The 1965 Committee has maintained interest in several of its past commitments in addition to current affairs. The following items are commended to the Association for adoption as the report of this Committee.

1. **Cooking of Garbage Fed to Swine.**

Regulatory officials of the various states are encouraged to continue vigorous enforcement of the garbage cooking requirements. Such enforcement is considered vital to the control of the spread of trichinosis, hog cholera, and other transmissible diseases of swine. An average of 7,000 hogs fed raw garbage are marketed per month from a total of 20 states.

2. **Artificial Insemination.**

The committee reaffirms its recommendations of 1964 concerning the health standards for boar studs. Furthermore, it is considered essential to the control of the spread of transmissible diseases through contact that the sale of boars from testing stations for breeding purposes be prohibited. There is much need for research on diseases which are transmissible by boars through natural service or through semen used in artificial insemination. Although some dangers are recognized there remains vast vagueness in the realities of the problems involved. In the interim diagnostic interests are encouraged to be alert to the need for recorded information on conditions of infectious nature which seem to be related to natural service or semen.

3. **Transmissible Gastroenteritis.**

Immunizing agents have begun being commercially available for use in the prevention of Transmissible Gastroenteritis (TGE). The evaluation of the efficacy of such vaccines is difficult under
existing laboratory means of diagnosis. The committee strongly urges the development of diagnostic criteria for this disease. In the full discussion of this matter it was considered that a useful means of accomplishing this task would be through the appointment of a sub-committee composed of members of this committee and a similar group from the Conference of Veterinary Laboratory Diagnosticians. Action has been taken by the Committee, subject to the approval of the Association, to create a sub-committee to study possible criteria for the diagnosis of TGE. The sub-committee will seek such counsel and information from knowledgeable persons in the nation as is useful to meet the requirements of their mission. In addition, in order to obtain information on morbidity and mortality among swine due to TGE, all state and regulatory officials are urged to use the means available to them to obtain this information.

These records will be of considerable importance in evaluating the impact of the disease on the swine industry, in the development of preventive measures, and in its eventual elimination.


The committee reaffirms its interest in the problem of jowl abscesses. Carcass condemnations due to this condition and resultant pyemia warrant action on the call of the swine industry for support of research to solve the problem so that preventive measures may be found that are effective.

5. Peri-natal Disease Research.

The need for the prevention and control of diseases of embryo and newborn pigs is accentuated by the intensive management practices applied by swine producers. The committee recognizes the need therefore to emphasize the value of strong support for research on the aspects of infectious diseases that effect embryos and very young pigs. Losses due to embryo death, infertility, and baby pig deaths retard the essential growth of the swine industry's efforts to provide food for the expanding population.
Hog cholera virus vaccines have been produced by biological modification (modified live virus vaccines) and physico-chemical inactivation (killed virus vaccines), both of which are qualitative changes. Torrey, in hog cholera virus titration studies, used increased doses of virus in a dilution series with results which indicated the development of partial immunity. This suggested the hypothesis that quantitative factors may be operative in virulence of hog cholera virus. The object of the preliminary and exploratory studies being reported here is to establish a foundation of experimental evidence for 1) further research on the immunizing effect of repeated, increasingly larger but sub-lethal doses of virulent hog cholera virus on swine, 2) demonstrate the variations in reactions in different swine associated with exposure or challenge with minimal infecting dosages of virus and 3) to present other usages of minimal infecting dosages of live virus in swine such as for virus characterization.

The observations reported here were made incidental to a series of virus titration studies over a period of two years. Since each virus titration study was an entity within itself and each titration was performed at a different time, with pigs from a different farrowing period or a different source, with a different specimen of virus and many other variable details, there was no total uniformity between any one of the studies and any other. For this reason, many seemingly inexplicable variations will be found in the subject animals and in the virus itself. These were due to the wide range of time involved in these experiments, the variation in management practices of the owners from whom the experimental animals were procured, but most importantly, each experiment was an individual titration and although it was consistent within itself, it was not consistent for uniformity with the other experiments in this report. Observations are reported and directions for further research are suggested but no positive or finite conclusions have been made. In accordance with standard experimental design, the pigs in each experiment were distributed among the differently exposed groups of each experiment so that available litter representation was relatively uniform in each exposure dosage group in order to preclude attribution of experimental results to litter differences.

REACTIONS OF PIGS TO DOSES OF VIRULENT HC VIRUS

MATERIALS AND METHOD

Virus - The virus used was lyophilized virulent BAI virus 7183 (1946), procured from Dr. L. O. Mott, National Animal Disease Laboratory, Ames, Iowa. This virus was received in three ampules, each ampule containing the end product of lyophilizing two ml. of hog cholera-infected blood. Reconstitution was accomplished by the addition of 10 ml. of sterile physiological saline solution.

Ampule number two was reconstituted as described above on 1/12/61. Ampule number one was not used in any of the work described herein. The entire contents of ampule number two was injected subcutaneously into the tissues of the axillary region of one nonvaccinated pig on the day of reconstitution, 1/12/61. On the seventh day after injection, 1/19/61, this pig was bled and two ml. of its blood was injected into each of two nonvaccinated swine. These swine were exsanguinated on the seventh day after injection, 1/26/61, the blood was defibrinated, bottled, identified as Station Hog Cholera Virus serial number (s.n.) 1b and stored at -70°F until used. Ampule number three was reconstituted on 11/6/62, treated in the same manner as described above for the preparation of station hog cholera virus and was identified as Station Hog Cholera Virus s.n. 1c on 11/20/62.

A dose of one ml. of undiluted virus was used as the final challenge to test for complete immunity to hog cholera. The Lethal Dose (LD) was calculated by the method of Reed and Muench. Postmortem examinations were made of all pigs which died during the course of the experiments. All swine which died exhibited lesions usually associated with hog cholera.

Degrees of immunity were considered at three levels; non-immune (susceptible), partially immune and completely immune. Pigs were considered to have been susceptible to hog cholera if after receiving the first administration of diluted virus they developed the clinical signs of hog cholera, died and showed lesions at necropsy as described by Dunne. Pigs were considered to be partially immune if they survived challenge with diluted virus at least one time and succumbed after a subsequent injection of a larger dose of virus. Pigs were considered to be completely immune if they survived challenge with one ml. of undiluted hog cholera virus either with or without reaction.

Experiment 2739 - This experiment was started on 5/3/62. The virus was Station Hog Cholera Virus s.n. 1b and was 462 days old and had been stored at -70°F. The pigs were crosses of Hampshire and Tamworth breeds. They were from two litters procured from one owner. The pigs were weaned at five weeks of age and initially exposed at 10 weeks of age. Their dams and the sire had been vaccinated with modified live virus vaccines two years previously.

One ml. of each of the following dilutions of Station Hog Cholera Virus s.n. 1b was given to each of two pigs: 10⁻⁶.⁷, 10⁻⁶.⁴, 10⁻⁶, 10⁻⁵ and 10⁻⁴. Forty-five days later, the survivors were given the next greater dose of virus in the series. This was continued at 45-day
intervals until either death occurred or a final dose of one ml. of the undiluted virus was given to the survivors.

Experiment 3037 - This experiment was started on 8/3/62. The virus was Station Hog Cholera Virus s.n. 1b. This was the same serial as was used in experiment 2739 except that it had been in -70°F storage for 554 days, from its preparation on 1/26/61 to the date of the commencement of this experiment on 8/3/62. Ten Hampshire pigs from two litters were procured from the same owner who supplied the pigs used in experiment 2739. The pigs from litter one were weaned at nine weeks of age and initially exposed at 12 weeks of age, whereas those from litter two were weaned at six weeks of age and initially exposed at nine weeks of age.

The procedure of experiment 3037 was the same as in experiment 2739 except for the dosages of the virus which were one ml. of each of the following dilutions: 10⁻⁶, 10⁻⁵.⁷, 10⁻⁵, 10⁻⁴.⁷ and 10⁻⁴, and the interval between injections was 30 days.

Experiment 3402 - This experiment was started on 12/7/62. The virus was Station Hog Cholera Virus s.n. 1c and was 17 days old and had been stored at -70°F. Eighteen Hampshire pigs from three litters were procured from the same owner who supplied the pigs used in experiments 2739 and 3037. The pigs in litters one and two were weaned at four weeks of age and initially exposed at 10 weeks of age. The pigs in litter three were weaned at three weeks of age and initially exposed at nine weeks of age.

The procedure of experiment 3402 was the same as experiment 3037 except for the dosages of the virus which were one ml. of each of the following dilutions: 10⁻⁶.⁷, 10⁻⁶.⁴, 10⁻⁶, 10⁻⁵.⁷, 10⁻⁵ and 10⁻⁴.

Experiment 4308 - This experiment was started on 4/7/64. The virus was Station Hog Cholera Virus s.n. 1c. This was the same serial as was used in experiment 3402 except that it had been in -70°F storage for 504 days, from its preparation on 11/20/62 to the date of the commencement of this experiment on 4/7/64. The Tissue Culture Infective Dose ₅₀ (TCID₅₀) was derived by immunofluorescence as developed by Mengeling et al.⁴ and Mengeling, Pirtle and Torrey.⁵ Twenty-four Hampshire pigs from three litters procured from two owners, one from Georgia and one from Florida, were used. Neither of these owners supplied the pigs for any of the previous experiments. The dams of the Florida pigs had not been vaccinated, whereas the dam of the Georgia pigs had been vaccinated within the last two years with killed virus vaccine. All the pigs were weaned at six weeks of age and initially exposed at eight weeks of age.

The procedure of experiment 4308 was the same as experiment 3402 except for the dosages of the virus which were one ml. of each of the following dilutions: 10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³, 10⁻² and 10⁻¹, and the interval between injections was 21 days.
RESULTS

Experiment 2739, Table IA - The calculated LD₅₀ of the virus used in this experiment was 10⁵.₀ per ml.

In the initial exposures (Tables IA and IB) three pigs (5752, 5753 and 5754) exhibited clinical signs of hog cholera an average of 11.3 days and died. They were considered to have been susceptible. Five pigs (5746, 5748, 5749, 5750 and 5751) survived either the first, second or third injections of diluted virus with slight, variable hog cholera reactions. These pigs, when given the next larger dose of virus, were sick an average of 19.8 days and succumbed. These pigs were considered to have been partially immune. Three of these pigs (5749, 5750 and 5751) had slight hog cholera reactions following the first injection of diluted virus but recovered. Upon the second subsequent exposure to a larger dose of virus, however, they developed the disease and died. The earlier hog cholera reaction of these pigs had not caused them to produce sufficient antibody to render them completely immune.

Experiment 3037, Table IIA - In this experiment, the LD₅₀ of the virus was calculated to be 10⁵.₇ ml.

In this series of exposures (Tables IIA and IIB) seven pigs (6198, 6200, 6202, 6204, 6205, 6206 and 6207) exhibited the usual clinical signs of hog cholera an average of 12.3 days before death and were considered to have been susceptible. Two pigs (6199 and 6201) survived the first and second injections of diluted virus although they suffered severe hog cholera reactions each time. These pigs were then given the next greater dose of virus, and had a severe hog cholera reaction an average of 16.5 days before death. They were considered to have been partially immune. One pig (6203) after being sick for 23 days and receiving successively greater doses of virus and finally the dose of one ml. of undiluted virus survived and was considered to be completely immune.

Experiment 3402, Table IIIA - The calculated LD₅₀ of the virus used in this experiment was 10⁴.₈ per ml.

In this experiment (Tables IIIA and IIIB), four pigs (6990, 6991, 6992 and 6993) had severe hog cholera reactions an average of 10.5 days and then died. These pigs were considered to have been susceptible. Nine pigs (6976, 6977, 6979, 6982, 6984, 6985, 6987, 6988, 6989) survived either the first, second, third or fourth injections of diluted virus without reaction. These pigs when given the next greater dose of virus, had hog cholera reactions an average of 15.2 days, the reactions terminating in death. These pigs were considered to have been partially immune. Five pigs (6978, 6980, 6981, 6983, 6986) received successively greater doses of virus and finally the one ml. dose of undiluted virus survived and were considered to have been completely immune.

Experiment 4308, Table IVA - As a result of the initial injection of virus in this experiment, it was determined that the calculated LD₅₀ was 10⁴.₇ per ml. and the TCID₅₀ was 10⁴.₆.


<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Source of Pigs</th>
<th>Injections at 45 Day Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Owner</td>
<td>Litter</td>
</tr>
<tr>
<td>5745</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>5746</td>
<td>A</td>
<td>2</td>
</tr>
<tr>
<td>5747</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>5748</td>
<td>A</td>
<td>2</td>
</tr>
<tr>
<td>5749</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>5750</td>
<td>A</td>
<td>2</td>
</tr>
<tr>
<td>5751</td>
<td>A</td>
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<td>5752</td>
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<td>5753</td>
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<td>1</td>
</tr>
<tr>
<td>5754</td>
<td>A</td>
<td>2</td>
</tr>
</tbody>
</table>

2. One ml. of the virus dilution was injected.
3. Line indicates calculated LD_{50} dose.
4. Imm. = Immune.
5. D = Died with symptoms and lesions at necropsy usually associated with hog cholera.
### TABLE IB
Summary of Results of Experiment 2739

<table>
<thead>
<tr>
<th>Reaction to Exposure to Virulent Virus</th>
<th>Susceptible</th>
<th>Partially Immune</th>
<th>Completely Immune</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number Pigs</td>
<td>Average Number of Days of Reaction</td>
<td>Number Pigs</td>
<td>Average Number of Days of Reaction</td>
</tr>
<tr>
<td></td>
<td>Number Percent</td>
<td></td>
<td>Number Percent</td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>3</td>
<td>30.0</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>Partially Immune</td>
<td>5</td>
<td>11.3</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>Completely Immune</td>
<td>2</td>
<td>19.8</td>
<td>2</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100.0</td>
<td>10</td>
<td>100.0</td>
</tr>
</tbody>
</table>
### TABLE IIA

**Experiment 3037 - Reactions of Pigs to Sub-lethal Doses of Virulent Hog Cholera Virus - Station Hog Cholera Virus s.n. 1b**

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Source of Pigs</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Owner</td>
<td>Litter</td>
<td>Virus Dilutions</td>
<td>Number of Days Reaction</td>
</tr>
<tr>
<td>6198</td>
<td>A 3</td>
<td></td>
<td>10^-6</td>
<td>13-D^3</td>
</tr>
<tr>
<td>6199</td>
<td>A 4</td>
<td></td>
<td>10^-6</td>
<td>15</td>
</tr>
<tr>
<td>6200</td>
<td>A 3</td>
<td></td>
<td>10^-5.7</td>
<td>15-D^3</td>
</tr>
<tr>
<td>6201</td>
<td>A 4</td>
<td></td>
<td>10^-5.7</td>
<td>19</td>
</tr>
<tr>
<td>6202</td>
<td>A 3</td>
<td></td>
<td>10^-5</td>
<td>18-D^3</td>
</tr>
<tr>
<td>6203</td>
<td>A 4</td>
<td></td>
<td>10^-5</td>
<td>23</td>
</tr>
<tr>
<td>6204</td>
<td>A 3</td>
<td></td>
<td>10^-4</td>
<td>13-D^3</td>
</tr>
<tr>
<td>6205</td>
<td>A 4</td>
<td></td>
<td>10^-4</td>
<td>13-D^3</td>
</tr>
<tr>
<td>6206</td>
<td>A 3</td>
<td></td>
<td>10^-4</td>
<td>14-D^3</td>
</tr>
</tbody>
</table>

2. One ml. of the virus dilution was injected.
3. D = Died with symptoms and lesions at necropsy usually associated with hog cholera.
4. Line indicates calculated LD_{50} dose.
5. Imm. = Immune.
<table>
<thead>
<tr>
<th>Susceptible</th>
<th>Partially Immune</th>
<th>Completely Immune</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pigs</strong></td>
<td><strong>Number</strong></td>
<td><strong>Percent</strong></td>
<td><strong>Number</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Average</strong></td>
<td><strong>Days of</strong></td>
<td><strong>Average</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Number</strong></td>
<td><strong>Reaction</strong></td>
<td><strong>Number</strong></td>
</tr>
<tr>
<td>4</td>
<td>22.2</td>
<td>10.5</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>50.0</td>
<td>15.2</td>
<td>18</td>
</tr>
</tbody>
</table>
TABLE IIIA
Experiment 3402 - Reactions of Pigs to Sub-lethal Doses of Virulent Hog Cholera Virus -
Station Hog Cholera Virus s.n. 1c

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Source of Pigs</th>
<th>Injections at 30 Day Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus Dilution2</td>
</tr>
<tr>
<td>6976</td>
<td>A 5</td>
<td>10^-6.7</td>
</tr>
<tr>
<td>6977</td>
<td>A 6</td>
<td>10^-6.7</td>
</tr>
<tr>
<td>6978</td>
<td>A 7</td>
<td>10^-6.7</td>
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<td>6979</td>
<td>A 5</td>
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</tr>
<tr>
<td>6980</td>
<td>A 6</td>
<td>10^-6.4</td>
</tr>
<tr>
<td>6981</td>
<td>A 7</td>
<td>10^-6.4</td>
</tr>
<tr>
<td>6982</td>
<td>A 5</td>
<td>10^-6</td>
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<tr>
<td>6993</td>
<td>A 7</td>
<td>10^-4</td>
</tr>
</tbody>
</table>

2. One ml. of the virus dilution was injected.
3. D = Died with symptoms and lesions at necropsy usually associated with hog cholera.
4. Line indicates calculated LD50 dose.
5. Imm. = Immune.
TABLE IIb
Summary of Results of Experiment 3037

<table>
<thead>
<tr>
<th></th>
<th>Susceptible</th>
<th>Partially Immune</th>
<th>Completely Immune</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pigs Number</td>
<td>Pigs Number</td>
<td>Pigs Number</td>
<td>Pigs Number</td>
</tr>
<tr>
<td></td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average Days of</td>
<td>Average Days of</td>
<td>Average Days of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reaction</td>
<td>Reaction</td>
<td>Reaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td>Susceptible</td>
<td>7</td>
<td>70.0</td>
<td>12.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Partially Immune</td>
<td>2</td>
<td>20.0</td>
<td>16.5</td>
<td>16.5</td>
</tr>
<tr>
<td>Completely Immune</td>
<td>1</td>
<td>10.0</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100.0</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>
The series of exposures (Tables IVA and IVB) showed that seventeen pigs (9098, 9104, 9105, 9107, 9108, 9110, 9111, 9112, 9113, 9114, 9115, 9116, 9117, 9118, 9119 and 9120) exhibited clinical signs of hog cholera and lesions at necropsy usually associated with hog cholera and were considered to have been susceptible. Their reaction lasted an average of 10.4 days before death, two of them (9098 and 9104) having received less than the calculated LD50 dose of the virus, the remaining 15 pigs having received more than the calculated LD50 dose. An interesting facet of the reactions of susceptible pigs occurred in this experiment. In the 15 pigs which died following the first injection with more than the calculated LD50 dose of virus, the average number of days of reaction varied directly with the size of the dose of virus. The dosages of virus consisted of 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} dilutions, and the average days of illness were 9.3, 10.5, 10.8 and 11.8, respectively.

Five pigs (9097, 9099, 9101, 9102, 9103) survived the first injection of diluted virus without reaction. When given the next greater dose, however, they reacted an average of 13.4 days and died. These pigs were considered to be partially immune.

Two pigs (6993, 9100) were considered to be completely immune after receiving progressively larger doses of virus and then surviving the final dose of one ml. of undiluted virus without reaction.

In the four experiments, 31 of 62 pigs or 50.0 percent died following the initial injection of virus doses. They had hog cholera reactions averaging 10.9 days and were considered to be susceptible (Table V). Of these 31 pigs, four (6198, 6990, 9098 and 9104) received less than the calculated LD50 dose of virus and reacted an average of 9.8 days, whereas the remaining 27 pigs received the calculated LD50 dose or more and reacted an average of 11.0 days.

Twenty-one pigs or 33.0 percent received two, three, four or five injections of diluted virus, had hog cholera reactions which terminated in death. These pigs were considered to be partially immune. Of these 21 partially immune pigs, six (6976, 6977, 6979, 6982, 9097, and 9099) were given a virus exposure of less than the calculated LD50 dose and they had hog cholera reactions an average of 14.3 days which terminated in death. The remaining 15 pigs had a virus exposure of more than the calculated LD50 dose and had reactions an average of 16.7 days which terminated in death.

Ten pigs or 16.1 percent received increasingly larger doses of virus and although they had hog cholera reactions, they survived a one ml. challenge dose of undiluted, virulent virus and thus were considered to be completely immune.

It should be noted that nine of the ten completely immune pigs exhibited no reaction following the administration of the one ml. challenge dose of undiluted virus. Pig number 6203 was the one exception. It had a reaction of nine days duration following the administration of one ml. of undiluted virus. Each of these 10 pigs, including number 6203, however, did have one previous reaction, the average of which was
TABLE IVA

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
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<tr>
<td></td>
<td>Virus</td>
<td>Dilution</td>
<td>Reaction</td>
<td>Virus</td>
<td>Dilution</td>
<td>Reaction</td>
</tr>
<tr>
<td>9097</td>
<td>G-B</td>
<td>10^{-4}</td>
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<td>10^{-5}</td>
<td>0</td>
<td>15-D3</td>
</tr>
<tr>
<td>9098</td>
<td>G-B</td>
<td>10^{-4}</td>
<td>0</td>
<td>10^{-4}</td>
<td>0</td>
<td>10^{-5}</td>
</tr>
<tr>
<td>9099</td>
<td>G-C</td>
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<td>0</td>
<td>10^{-6}</td>
<td>0</td>
<td>10^{-6}</td>
</tr>
<tr>
<td>9100</td>
<td>G-B</td>
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<td>0</td>
<td>10^{-4}</td>
<td>0</td>
<td>10^{-4}</td>
</tr>
<tr>
<td>9101</td>
<td>G-C</td>
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<td>0</td>
<td>10^{-6}</td>
<td>0</td>
<td>10^{-6}</td>
</tr>
<tr>
<td>9102</td>
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<td>9103</td>
<td>F-C</td>
<td>9</td>
<td>10^{-5}</td>
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<td>10^{-5}</td>
<td>0</td>
</tr>
<tr>
<td>9104</td>
<td>F-C</td>
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<td>10^{-5}</td>
<td>0</td>
<td>10^{-5}</td>
<td>0</td>
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<tr>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>9113</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>10^{-5}</td>
<td>0</td>
<td>10^{-5}</td>
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</tr>
<tr>
<td>9119</td>
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<td>10^{-5}</td>
<td>0</td>
<td>10^{-5}</td>
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</tr>
<tr>
<td>9120</td>
<td>F-C</td>
<td>9</td>
<td>10^{-5}</td>
<td>0</td>
<td>10^{-5}</td>
<td>0</td>
</tr>
</tbody>
</table>

1. **G** = Georgia, **F** = Florida, **B, C** = Code letters for owner's name
2. **D** = Died with symptoms and lesions at necropsy usually associated with hog cholera.
3. **L** = Indicates calculated LD50 dose.
4. **Imm.** = Immune.
### TABLE IVB
Summary of Results of Experiment 4308

<table>
<thead>
<tr>
<th>Reaction to Exposure to Virulent Virus</th>
<th>Susceptible</th>
<th>Partially Immune</th>
<th>Completely Immune</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>70.8</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td></td>
<td>13.4</td>
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</tbody>
</table>

Number of Pigs: 24
Percent: 100.0%
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<tr>
<th>Experiment</th>
<th>Pigs Number</th>
<th>Percent</th>
<th>Average Number of Days of Reaction</th>
<th>Pigs Number</th>
<th>Percent</th>
<th>Average Number of Days of Reaction</th>
<th>Pigs Number</th>
<th>Percent</th>
<th>Average Number of Days of Reaction</th>
<th>Total Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2739</td>
<td>3</td>
<td>30.0</td>
<td>11.3</td>
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<td>12.5</td>
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<td>100.0</td>
</tr>
<tr>
<td>3037</td>
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<td>70.0</td>
<td>12.3</td>
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<tr>
<td>3402</td>
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<td>15.2</td>
<td>5</td>
<td>27.8</td>
<td>9.2</td>
<td>18</td>
<td>100.0</td>
</tr>
<tr>
<td>4308</td>
<td>17</td>
<td>70.8+</td>
<td>10.4</td>
<td>5</td>
<td>20.8+</td>
<td>13.4</td>
<td>2</td>
<td>8.3+</td>
<td>16.0</td>
<td>24</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
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<td>21</td>
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</tr>
<tr>
<td>Average</td>
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<td>16.0</td>
<td></td>
<td></td>
<td>12.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE V
Comparison and Summary of Exposure to Virulent Virus
12.3 days. It should also be noted that two of these pigs (5745 and 9100) had their reactions after doses of virus below the calculated LD_{50} dose and eight of them (5747, 6203, 6978, 6980, 6981, 6983, 6986 and 9106) were above that dose.

**DISCUSSION**

The particulate morphology of virus suggests the possibility that its degree of pathogenicity may depend on quantitative as well as qualitative factors. Since virus may be immunogenic without being pathogenic, it may be hypothesized that virus may be antigenic without being immunogenic. The antigenicity of a virus may be of such low grade that it would cause the pig to produce only enough antibody to be partially immune - antigenic, but not produce enough antibody to be completely immune - immunogenic. Upon successive exposures to increasingly larger doses of virus, however, the cumulative antigenic action of the virus would cause the pig to produce enough antibody for complete immunity. Since the complete immunization of ten pigs in the above described experiments was accomplished with sub-lethal doses of virulent virus, and each of the ten pigs had a hog cholera reaction and recovered prior to the final challenge with one ml. of undiluted virus, it would seem that the production of this complete immunity was of a quantitative nature as well as being qualitative. In other words, a minimum number of virus particles was necessary to produce disease in addition to the virus itself being virulent.

Although all 10 completely immune pigs had at least one reaction and recovered prior to the administration of the one ml. dose of undiluted virus, there were five pigs that also had reactions and recovered but were not completely immune. The cholera reactions in these pigs did not indicate a hog cholera immune response.

Another aspect of these results is that they may be due to varying concentrations of the soluble antigen fraction and the infective fraction in different serials of hog cholera virus. This suggests the possibility that these fractions dilute out at different levels, and that therefore different degrees of immunity would be manifested at different dilution levels in different samples of virus. More work needs to be done to study this aspect of the characterization of hog cholera virus.

**SUMMARY**

Of 62 pigs, 31 died following initial exposure to virulent hog cholera virus. The other 31 pigs survived the initial exposure to hog cholera virus and were given increasingly larger but sub-lethal doses.

Twenty-one pigs survived the initial exposure and one or more subsequent exposures but did not develop complete immunity. Upon exposure to greater doses of virus they developed hog cholera and died. Five of these 21 pigs had hog cholera reactions following doses of diluted virus and recovered. Upon challenge with larger doses of virus,
however, they were found not to have developed complete immunity. In other words, a hog cholera reaction did not necessarily indicate an immune response.

Ten pigs survived the initial exposure and subsequent exposures to diluted but increasingly larger doses of virus and developed complete immunity. This was demonstrated by their survival following challenge exposure to one ml. of undiluted hog cholera virus.

Hog cholera virus in order to be pathogenic for swine seemed to require a quantitative minimum (particulate) as well as a qualitative minimum (virulence) of virus.

REFERENCES

An international meeting to discuss hog cholera and African swine fever was held in Rome during June 1965, sponsored by the Food and Agriculture Organization of the United Nations (FAO) and the International Office of Epizootics (OIE). More than 100 veterinarians and other scientists from 42 countries attended this meeting, with representation also from the sponsoring organizations, the European Economic Community, and the World Veterinary Congress.

It was evident that hog cholera, so long of prime concern to us here in the United States, also continues to be of significance in other parts of the world. To date, although several countries have succeeded in eradicating the disease, most must continue to face the dangers to their swine industries associated with the continued presence of the disease. In addition to Australia, Canada, Denmark, Ireland, and Norway—which have eradicated hog cholera—several other countries—including France, the United Kingdom, the Netherlands, and the United States—have initiated programs leading to eradication. All are somewhat further along than this country, being engaged in stamping out efforts involving slaughter of infected and exposed animals. There is also a great deal of study being devoted to hog cholera and its possible eradication in many other countries.

It was generally recognized during the discussions that safer hog cholera vaccines must be sought. It was pointed out that most countries that had achieved, or nearly achieved, eradication did not use live virus vaccines. Conversely, the importance of vaccination in earlier stages of a program as an aid in reducing incidence was recognized. Many countries reported an apparent increase in chronic, or "inapparent," forms of the disease, although it was emphasized that the acute form is still in plentiful supply. Several countries where this low-grade form of the disease is bothersome have never used attenuated live virus vaccines, which is interesting in view of the comment that has been heard in this country that less acute field outbreaks must be due to one of the vaccine viruses.

Diagnosis of hog cholera was discussed at length. The group emphasized the importance of the veterinarian in the field in making the diagnosis, particularly with the more obscure types of the disease now being uncovered. It was also stressed that the field veterinarian must be supported by all aids available, including the laboratory.

Eradication was defined as elimination of the disease and the need to vaccinate against it. This is in close agreement with definitions used in this country for the hog cholera eradication program, as well as others.

*Director, Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture.
African swine fever was reviewed at some length. It was stated that this disease is truly an emergent disease of the twentieth century, brought about accidentally when the balance between the natural hosts (African wild swine) and the virus was upset by the introduction of an alternate host (domestic swine). Failure to eradicate this new disease led in turn to the establishment of a new cycle in domestic pigs, which recent evidence from Spain indicates is working toward the type of virus-host balance present in Africa before domestic swine became involved.

With this changing pattern, the carrier state of the virus in domestic swine becomes most significant in pathogenesis of the disease. It has been demonstrated that "carrier" animals are not consistently infectious, and may not become so until the latent virus is activated by stress of one type or another. Also, the character of clinical signs of African swine fever has changed in areas where the disease has become endemic in domestic swine. Much milder forms are now observed with corresponding change in lesion patterns, making it increasingly difficult to differentiate between hog cholera and African swine fever in the field. In the laboratory, even with the changing pattern of the disease, the hemadsorption test has remained efficacious; although specimens from less acute field cases may require additional passage in leucocyte cultures before a positive result is obtained.

Another major finding has been the Spanish report of tick transmission of African swine fever virus, with the virus surviving in the ticks (Ornithodoros) for one year or longer. Ability of such ticks to transmit the virus was also reported from Africa.

Eradication in Africa, with the virus being perpetuated in species of wild swine, is presently impossible, although the disease can be prevented in domestic swine by strict confinement behind double fences. Eradication in Europe is made difficult by the presence of free-ranging domestic pigs. An important part of the control effort in Spain is an information and education program to persuade swine producers to take their pigs from the range and use confinement methods of husbandry. Great credit was given by the delegates to Spanish scientists for their help to the rest of Europe through work on African swine fever.

The experience of France with the disease is also of interest to us in the United States, as the French have apparently been successful in eliminating African swine fever even in the face of the simultaneous existence of hog cholera. This was accomplished by prompt and drastic livestock sanitary measures, including the immediate slaughter of all suspect sick and exposed swine in all cases resembling either hog cholera or African swine fever, without waiting for differential diagnosis. As no practical and effective vaccine has yet been developed for field use in African swine fever, the experience gained in other countries in fighting this disease becomes of urgent interest to us in this country. We may be confronted with the same situation at any time.

Certain recommendations were made by the delegates at the close of the meeting. These are outlined as follows:
1. All countries should declare African swine fever and hog cholera to be reportable diseases, with the customary notification to OIE and neighboring countries.

2. Veterinary services of all countries in which either of these diseases occur should intensify their studies on the epizootiology of the diseases.

3. A comprehensive study, by appropriate international agencies, should be undertaken on the African continent to determine the incidence of African swine fever and the distribution of virus reservoirs.

4. Due to the progress in development of laboratory aids in diagnosis of African swine fever and hog cholera, FAO and OIE should designate reference laboratories for supplying standard diagnostic reagents.

5. Whenever a swine fever-like disease is diagnosed in a country where African swine fever is enzootic, and in adjacent countries, an immediate program of destruction of all suspect sick and exposed pigs should be initiated, together with other hygienic measures, without waiting for differential diagnosis.

6. The use of virulent hog cholera virus or inadequately attenuated vaccines should be prohibited. Use of inactivated, or attenuated viruses of low or no pathogenicity, is recommended.

7. The feeding of swill and animal by-products should be prohibited absolutely. If this is not possible, then it must be sterilized under veterinary supervision.

8. The movement of pigs and pig products should be under strict veterinary control.

9. Fundamental studies on the viruses of hog cholera and African swine fever, as well as reservoirs and vectors, should be encouraged.

10. The trend toward global eradication of hog cholera should be encouraged and the example of those countries which have eradicated the disease should be followed.
FURTHER EVALUATION OF HOG CHOLERA IMMUNIZING AGENTS BOVINE VIRUS DIARRHEA AND HOG CHOLERA VACCINE, MLV, TCO

T. W. Tamoglia, DVM; A. L. Tellejohn, DVM; C. E. Phillips, DVM; F. B. Wilkinson, B.Sc., DVM

INTRODUCTION

The 1964 report from the Committee on the Nationwide Eradication of Hog Cholera, United States Livestock Sanitary Association, states in part, "It appears that bovine virus diarrhea (BVD) vaccine has successfully withstood challenge from field strains of hog cholera virus to which it has been subjected." It then asks the question, "Is a product needed which is capable of withstanding challenge by field strains, but perhaps not capable of withstanding challenge by the standard Ames strain?"

Earlier studies in both specific pathogen free (SPF) pigs and field pigs indicated that experimental BVD vaccine had some merit for immunizing pigs against hog cholera, but was not consistently effective against all strains (isolates) of field hog cholera (HC) virus. Because of the increased general interest in the heterotypic vaccine concept for protection against a homotypic virus and the potential usefulness of this type of vaccine in the National Hog Cholera Eradication Program, additional studies reported here were conducted by the Veterinary Biologics and the Animal Health Divisions of the United States Department of Agriculture. The purpose was to (1) learn more about the use of BVD virus vaccine for the protection of pigs against several field isolates of HC virus, and (2) compare it with a modified live virus, tissue culture origin hog cholera vaccine (HCV).

Hog cholera susceptible pigs were vaccinated with the New York-1 strain of BVD virus, prepared as a live virus vaccine. Additional pigs originating from the same sources were vaccinated with a hog cholera modified live virus, tissue culture origin, vaccine to compare the results.

MATERIALS AND METHODS

Bovine Virus Diarrhea virus strain New York-1 (N.Y.-1) isolated from the spleen of a calf and supplied by Dr. J. A. Baker, Virus Research Institute, Cornell University, Ithaca, New York, was used in the vaccine.

From the Veterinary Biologics Division, United States Department of Agriculture, Agricultural Research Service: *Dr. Tellejohn is Senior Staff Veterinarian, Technical Analysis, Hyattsville, Maryland. Dr. Tamoglia is Biologics Veterinarian, Field Headquarters, Veterinary Biologics Division, Ames, Iowa. Dr. Phillips is Biologics Veterinarian, Laboratory Head, Large Animal Products, National Animal Disease Laboratory, Ames, Iowa. Dr. Wilkinson is BVD Staff Veterinarian, Kansas City, Missouri.

*Al Tellejohn died 1/2/66.
Virus from the third tissue culture passage was inoculated into primary bovine kidney tissue culture cells with Eagle's medium without serum and incubated seven days at 37°C. The titer of the finished vaccine prepared by the addition of a stabilizer-extender and lyophilized was $10^{4.6}$ per one ml or approximately 79,500 TCID$_{50}$ per two ml dose. This vaccine was prepared by the Jensen-Salsbery Laboratories, Division of Richardson-Merrell, Inc., Kansas City, Missouri.

A tissue culture origin, modified live virus hog cholera vaccine prepared under a United States Veterinary License was used in parallel tests to compare the results.

The HC viruses used for challenge in the testing were from ten sources. Eight field strains (isolates) were obtained from the Animal Health Division Diagnostic Laboratory, NADL, Ames, Iowa. These were isolated from tissues submitted to the laboratory, for diagnostic studies, from outbreaks in different locations throughout the United States. The two other strains used were the Veterinary Biologics Division (AI&-1) strain HC challenge virus and the (Baker) A-Strain HC virus.

We used 320 HC susceptible pigs in the weight range of 80-95 lb. in these tests. They were divided into twenty groups of sixteen pigs each. The pigs in each test were from the same farm, and in most cases the pigs in both of the paired tests (BVD and HCV) were from the same farm. Ten groups of pigs were used to test the BVD virus vaccine, and ten groups used to test the HC vaccine, MLV, TCO. They were arranged for convenience of challenge so that one group of BVD vaccinates with controls could be challenged with the same HC virus. Of the sixteen pigs in each test group, ten, selected at random, were vaccinated subcutaneously in the left axillary area with two ml of the reconstituted vaccine. The remaining six pigs were used as controls to test the susceptibility of the pigs and the virulence of the viruses used. Three of the control pigs remained in contact with the vaccinated pigs from the time of vaccination until challenge, while the other three were removed to isolated holding pens until the time of challenge. After challenge the sixteen pigs in each test were held together in the same pens.

Anti-hog cholera serum was not used with the vaccines. The immunizing or pre-challenge period (vaccination to challenge) was two weeks. The post-challenge period (challenge to end of test) was also two weeks.

The test pigs were observed daily and rectal temperatures taken and recorded when a pig appeared sick or slow. Clinical signs were recorded as: slow (x) slight anorexia, general appearance good, temperature not over 104°F.; or as sick (s) loss of appetite, diarrhea and/or vomiting, wobbling gate, temperature above 104°F. Dead pigs were examined for gross lesions of hog cholera, or other possible causes of death.

The rules for judging results of the tests were those used for judging potency test results of licensed hog cholera vaccines, modified live virus, and hog cholera vaccines (inactivated virus). The major differences in the rules for judging the test results of these two types of vaccine are as follows:
FURTHER EVALUATION

Modified live virus Hog Cholera Vaccine - At least 80 percent of the vaccinated pigs must survive challenge without showing visible signs of sickness.

Inactivated Virus Hog Cholera Vaccine - At least 80 percent of the vaccinated pigs must be alive and well at the conclusion of the test, and at least 50 percent must remain well throughout the test period.

In this project, when four of six control pigs became sick or died with the clinical syndrome indicated of hog cholera, it was considered sufficient evidence of susceptibility of the pigs used and virulence of the challenge virus. If less than four of the control pigs developed hog cholera following challenge, the test was judged a "no test." A few extra pigs from each source were obtained, for additional tests of susceptibility before starting the project, and in each case, hog cholera susceptibility was demonstrated.

RESULTS AND DISCUSSION

Of the ten BVD vaccine tests judged by Standard Requirement (SR) V-6 rules, one was satisfactory; two were "no test"; and seven were unsatisfactory. Of the ten comparative Hog Cholera Vaccine, MLV, TCO tests, nine were satisfactory, and one was a "no test." One of eight, or 13 percent, of the valid tests were satisfactory for the BVD vaccine. Judging the BVD vaccine tests by the rules applied for testing inactivated virus HC vaccines, SR-7, there were three satisfactory; two were "no test"; and five were unsatisfactory. Three of eight, or 38 percent, of the valid tests were satisfactory. The results of the Hog Cholera Vaccine, MLV, TCO tests were the same by both rules of judging, i.e., nine satisfactory, and one "no test." Nine of nine—100 percent of the valid tests were satisfactory. (Table I)

<table>
<thead>
<tr>
<th>Challenge Virus</th>
<th>Bovine Virus Diarrhea Vac SR V-6</th>
<th>SR V-7</th>
<th>Hog Cholera Vac SR V-6 or SR V-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Strain</td>
<td>Sat</td>
<td>Sat</td>
<td>Sat</td>
</tr>
<tr>
<td>AIQ Strain</td>
<td>Unsat</td>
<td>Unsat</td>
<td>Sat</td>
</tr>
<tr>
<td>V-210 Isolate</td>
<td>Unsat</td>
<td>Unsat</td>
<td>Sat</td>
</tr>
<tr>
<td>V-204 Isolate</td>
<td>Unsat</td>
<td>Sat</td>
<td>Sat</td>
</tr>
<tr>
<td>V-243 Isolate</td>
<td>Unsat</td>
<td>Sat</td>
<td>Sat</td>
</tr>
<tr>
<td>V-155</td>
<td>No test</td>
<td>No test</td>
<td>Sat</td>
</tr>
<tr>
<td>V-208</td>
<td>No test</td>
<td>No test</td>
<td>No test</td>
</tr>
<tr>
<td>V-139</td>
<td>Unsat</td>
<td>Unsat</td>
<td>Sat</td>
</tr>
<tr>
<td>V-315</td>
<td>Unsat</td>
<td>Unsat</td>
<td>Sat</td>
</tr>
<tr>
<td>V-225</td>
<td>Unsat</td>
<td>Unsat</td>
<td>Sat</td>
</tr>
<tr>
<td>Totals</td>
<td>1 Sat, 2 N.T.</td>
<td>3 Sat, 2 N.T.</td>
<td>9 Sat, 1 N.T.</td>
</tr>
<tr>
<td></td>
<td>7 Unsat</td>
<td>5 Unsat</td>
<td>9/9 = 100 %</td>
</tr>
</tbody>
</table>

Sat - Satisfactory
N.T. - No test
An interesting comparison is made of the total number of vaccinated pigs in the twenty vaccine tests. Of 99 Bovine Virus Diarrhea vaccinates, 38 became sick following challenge; of these 23 died and 13 recovered; two were sick at the conclusion of the tests; 61 remained well throughout the post-challenge test period; a total of 74 were well at the conclusion of the tests. Of the 100 Hog Cholera Vaccine, MLV, TCO vaccinates, all remained well throughout the post-challenge period. (Table II)

**TABLE II**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BVD</td>
<td>100</td>
<td>99*</td>
<td>38</td>
<td>23</td>
<td>13</td>
<td>61</td>
<td>2</td>
</tr>
<tr>
<td>HCV</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

*One Vaccinated pig died before challenge.

The response in control pigs to all strains of the Hog Cholera viruses used for challenge disclosed that 87 percent became sick and 83 percent died in the BVD Vaccine contact controls, while 59 percent became sick and 55 percent died in the HC Vaccine contact controls. This would appear to be caused by the HC vaccine virus spreading from the vaccinates to the contact controls during the immunization period, because in the controls

**TABLE III**

Response to HC Virus Challenge in Control Pigs Used for Testing BVD Vaccine and Hog Cholera Vaccine, MLV, TCO

<table>
<thead>
<tr>
<th>Challenge Virus</th>
<th>-</th>
<th>Contact Controls</th>
<th>Isolation Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BVD VAC</td>
<td>HC VAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S   D   W</td>
<td>S   D   W</td>
</tr>
<tr>
<td>V-225</td>
<td>3*</td>
<td>2    0</td>
<td>1    1</td>
</tr>
<tr>
<td>V-315</td>
<td>3</td>
<td>3    0</td>
<td>2    2</td>
</tr>
<tr>
<td>V-139</td>
<td>3</td>
<td>3    0</td>
<td>3    3</td>
</tr>
<tr>
<td>V-208</td>
<td>1</td>
<td>1    2</td>
<td>1    1</td>
</tr>
<tr>
<td>V-155</td>
<td>1</td>
<td>1    2</td>
<td>2    1</td>
</tr>
<tr>
<td>V-243</td>
<td>3</td>
<td>3    0</td>
<td>3    3</td>
</tr>
<tr>
<td>V-204</td>
<td>3</td>
<td>3    0</td>
<td>1    1</td>
</tr>
<tr>
<td>V-210</td>
<td>3</td>
<td>3    0</td>
<td>2    2</td>
</tr>
<tr>
<td>AIQ</td>
<td>3</td>
<td>3    0</td>
<td>(0)  0</td>
</tr>
<tr>
<td>A Strain</td>
<td>3</td>
<td>3    0</td>
<td>2    2</td>
</tr>
<tr>
<td>Totals</td>
<td>26</td>
<td>25   4</td>
<td>17   (16)</td>
</tr>
</tbody>
</table>

Sick 87% 59% 97% 100%
Died 83% 55% 87% 97%

*One pig sick at conclusion of test  S - Sick
**One pig died before challenge  D - Died
W - Well
FURTHER EVALUATION

held separately until challenged, 100 percent became sick, 97 percent died, and one pig recovered. In comparison, 97 percent of the isolation controls in the BVD vaccine tests became sick after challenge, and 87 percent died. The percentage of deaths in the contact and isolation controls of the BVD tests were 83 percent and 87 percent respectively. (Table III)

SUMMARY AND CONCLUSIONS

Special tests were conducted using Bovine Virus Diarrhea Vaccine, New York-1 strain, to protect pigs against challenge with field isolates of hog cholera viruses and compare the effectiveness of it with Hog Cholera Vaccine, modified live virus, tissue culture origin.

The results demonstrate that while BVDvirus vaccine has some value in protecting pigs against challenge with field isolates of hog cholera viruses, it will not protect as well as the modified live virus hog cholera vaccine tested, nor meet the present United States Department of Agriculture testing requirements for release of either the modified live virus vaccines, or the inactivated Hog Cholera Vaccines.

REFERENCES

4. Standard Requirement for Hog Cholera Vaccine, Modified Live Virus, V-6, issued by the Veterinary Biologics Division, ARS, USDA.
5. Standard Requirement for Hog Cholera Vaccine, Inactivated Virus, V-7, issued by the Veterinary Biologics Division, ARS, USDA.
SWINE FEVER

The Eradication Programme in Great Britain 1963 to 1965

A. D. Campbell*

INTRODUCTION

Swine fever has been present in Great Britain for something more than a hundred years and in March, 1963, an official programme of eradication based on the slaughter with compensation of affected herds was introduced. This paper outlines the history of the disease in Great Britain and of the attempts at its eradication and control prior to 1963. The progress which has been made during the two years of the present campaign is then described.

HISTORY OF THE DISEASE

Much of the information on the history of swine fever suggests that its first appearance in the British Isles was about 1862. One of the earliest descriptions of the disease in British literature is by Budd (1965) who delivered a lecture on "Typhoid Fever in Pigs" to a meeting of the Council of the Royal Agricultural Society of England. Budd described the disease as having been the cause of heavy mortality in pigs in various parts of the kingdom and said that he had first heard of its occurrence in Scotland in a letter he had received in August, 1964 from Professor John Gamgee of the Veterinary School in Edinburgh who, he said, described to him a fatal outbreak of typhoid fever which had occurred among pigs in that city.

From 1865 the disease spread throughout the kingdom unchecked by any regulatory measures until 1878 when the "Typhoid Fever of Swine Order" was passed into law. This made the disease notifiable and gave local authorities discretionary powers to slaughter affected herds. These powers were not uniformly applied and in 1893 the Board of Agriculture itself embarked on an endeavour to stamp out the disease. During this campaign all ailing pigs and those in close contact with them were slaughtered on premises where the existence of the disease was confirmed and in 1908, in the hope of achieving better control, breeding pigs on the affected farms were also slaughtered. It is interesting to note that this additional precaution was introduced because it had been observed by Stockman (1907) that young pigs which had been born to sows which had recovered from the disease some months before, often succumbed to the disease. During these attempts at eradication by stamping-out, salvage of carcasses was the rule and, as we now know, must have contributed to a perpetuation of the disease.

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By 1915 it had become evident that eradication by stamping-out would not be achieved and the policy was abandoned in 1916 in favour of a policy of control aimed at reducing mortality and minimising spread. The use of serum was encouraged and serum/virus immunisation was allowed. Serum was supplied free of charge for use in affected herds but it proved to be of very doubtful value and its use was discontinued in 1922. Between that time and 1963 official policy was directed towards achieving a measure of control of the disease by maintaining all affected herds in quarantine until the disease had run its course. A continuous period of 56 days without illness or death attributable to swine fever was the accepted criterion of recovery of the herd. During the period of quarantine animals which appeared to be healthy were allowed to be removed on permit for immediate slaughter, and this undoubtedly contributed to a continuation of the disease in swill-fed herds. In districts where the disease became prevalent, control of movement and marketing was enforced.

Crystal violet vaccine was introduced in 1947 and an official registered vaccinated herd scheme was begun in December, 1953. Although the vaccine was widely used it was never employed on a scale which was likely to effect any substantial reduction in the national incidence of the disease. In 1962 it was estimated that of an annual output of approximately eleven million pigs, about one and a quarter million were vaccinated. Field trials with modified (lapinised) vaccine were carried out during 1954 and 1955 in a number of herds which were judged to be free from swine fever. It was found that in herds where the standard of husbandry was not good, quite severe losses occurred in young pigs after vaccination, and spread of the vaccine virus to unvaccinated contacts was demonstrated. It was clear that lapinised vaccine would introduce a complication in the interpretation of laboratory diagnostic procedures and after full consideration of these facts it was decided in 1957 not to issue the vaccine for general use and to cease production.

THE CURRENT ERADICATION PROGRAMME

The present policy of eradication by stamping-out was introduced on the 11th March, 1963. On that day there were 264 herds under restrictions because the existence of swine fever had been confirmed in them during the previous 56 days. Because the incubation period of the disease can be as long as 28 days it was decided to slaughter all the quarantined herds in which swine fever had been confirmed during the previous 28 days (these being the herds in which the disease was most likely to be still active). In consequence, all the pigs in 155 of the 264 herds were slaughtered. The existence of swine fever was subsequently reconfirmed in 14 of the other quarantined herds and these were slaughtered. The 95 remaining herds were eventually released from quarantine after a continuous period of 56 days freedom from evidence of the disease; six of these herds later developed swine fever and were slaughtered.

The monthly incidence curve during the period 11th March 1963 to 31st March 1965 is shown in Figure 1. Figures 2 to 5 show the national
distribution of the disease during this time in four approximately equal periods.

As has been mentioned, swine fever had been enzootic in Great Britain for over 100 years and when the stamping-out programme was introduced, the incidence of the disease was high and its distribution widespread. During the first six months the highest incidence was in those areas of the country where it had always been most prevalent.

Figure 1. Density of Pig Population - Great Britain.
Swine Fever Eradication - Great Britain

Number of Outbreaks:
- NIL.
- 1-5
- 6-10
- 11-20
- OVER 20

Figure 3
Swine Fever Eradication - Great Britain
Incidence and Distribution of Outbreaks: 1st. April - 30th. Sept. 1964

Figure 4
Swine Fever Eradication - Great Britain
Incidence and Distribution of Outbreaks 1st Oct, 1964 - 31st March, 1965

Figure 5
During the second and third six-monthly periods of the programme there was a rather similar geographical distribution of the disease but at a progressively reducing level of incidence. However, during the fourth six-monthly period (October 1964 to March 1965) large areas of Great Britain remained free from the disease, including the whole of Scotland; ten of the thirteen Welsh counties and twenty-three of the forty-six English counties. In fact only three counties in the whole country showed a high incidence during this period.

DIAGNOSIS

In the early stages of the programme, diagnosis did not present any major difficulty as in most of the cases the disease was already well established in the herd before being reported and affected animals showed characteristic clinical symptoms and post-mortem lesions. Later, however, more and more of the swine fever cases investigated were either in the early stages of acute disease or showed comparatively mild clinical illness with low mortality which did not immediately arouse suspicion of swine fever. It seems likely that before the introduction of the stamping-out policy, much of this mild disease went unreported and unrecognized.

It is interesting to note that during the first year, 80.4 percent of positive cases were diagnosed on clinical and gross post-mortem findings only and 19.6 percent with the aid of laboratory tests, whereas during the second year the comparable figures were 55.6 percent and 44.4 percent and during the last three months of that period only 27.6 percent of swine fever cases were diagnosed on clinical and gross post mortem findings and 72.4 percent with the aid of laboratory tests.

In the beginning, laboratory support was mostly from the gel diffusion precipitin test and histo-pathological examination of the brain. Both these aids to diagnosis were, however, of limited value in acute disease with death following a very short period of illness and in view of these limitations fluorescent antibody technique, using spleen tissue from febrile pigs, was introduced to assist in early diagnosis of such cases. Use has also been made of leucocyte counts, and biological tests with immune and susceptible pigs have been utilised much more freely than in the early stages of the programme. It became apparent quite early in the campaign that sows which were exposed to swine fever virus during pregnancy would make the task of eradication more difficult since these animals often remained clinically normal until the termination of pregnancy when they became infective and often gave birth to dead, mummified or live weakly piglets in the litter. Live piglets born to such sows often showed congenital tremor.

Congenital tremor in baby pigs has been regarded as associated with swine fever virus when hypoplasia of the cerebellum of the piglet, as described by Harding et al. (1964) could be demonstrated in addition either to the demonstration of antibody in the serum of the dam by gel diffusion precipitin test or, to the unvaccinated dam resisting challenge.

Observations made in the field have led to these opinions:—
1. That susceptible pigs of different ages vary in their reaction to swine fever virus, mature pigs being relatively resistant;
2. that the great variation which is seen in the degree of illness and mortality in different herds suggests a variety of virus strains;
3. that the clinical symptoms and gross post mortem findings in early acute swine fever and in the milder forms of the disease are not always of themselves diagnostic of swine fever;
4. that most errors of diagnosis can be avoided by employing a full range of laboratory aids and not placing too much reliance on clinical and gross post-mortem findings only.

ORIGIN OF INFECTION

The discovery of the source of infection is a pre-requisite of ultimate success in any programme of eradication by stamping-out and failure to uncover the true source of infection is likely to result in persistence of the disease. The longer the disease has existed in a herd before notification the more difficult is it likely to be to uncover the true origin of infection.

For the purpose of classification origins are described as "primary," that is, either obscure or possibly originating from swill, and "secondary," that is, arising from a known source of infection.

Because of the heavy weight of infection existing in the country in the early stages, a satisfactory explanation of origin was not reached in a very high proportion of cases. As the incidence was progressively reduced it was possible to devote more time to a detailed investigation of each outbreak, and this resulted in a marked improvement in the percentage of cases in which a precise origin was found (Figures 6 and 7) and in the discovery of a large proportion of secondary cases before the illness had reached the stage where the owner's suspicion of swine fever has been aroused.

An encouraging fact which emerges from an analysis of the origins is the striking reduction in the number of outbreaks in which the possibility of infection from swill was believed to exist. One hundred and forty outbreaks or 12.3 percent of all cases were placed in this category in 1963-64, but only 22 outbreaks or 7.1 percent of all cases were classed in this way in 1964-65. The last case of this type occurred in November, 1964, when by extensive tracing it was found that part of the swill was likely to have been derived from pigs, sent for slaughter from a herd in which the existence of swine fever was confirmed very shortly after their removal. The marked reduction in the swill hazard is regarded as supporting the view that a substantial reduction in the national weight of infection has been achieved. It is also regarded as support for the conclusion that swine fever infection is not being imported in pig meat. To support the policy of swine fever eradication imports of pig meat are allowed only from countries which are free from the disease or which are actively pursuing a policy of eradication and can satisfy the veterinary safeguards which are laid down.
CLASSIFICATION AND ORIGINS OF OUTBREAKS

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<tr>
<th></th>
<th>HALF-YEAR</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Secondary</td>
<td>Primary</td>
<td>Secondary</td>
<td>Primary</td>
<td>Secondary</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>11.3.63-30.9.63</td>
<td>414</td>
<td>48.3</td>
<td>443</td>
<td>51.7</td>
<td>557</td>
<td>49.1</td>
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<td>23.0</td>
<td>110</td>
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Figure 6

<table>
<thead>
<tr>
<th></th>
<th>1963-64</th>
<th>1964-65</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
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<tr>
<td>PRIMARY CASES</td>
<td>557</td>
<td>49.1</td>
</tr>
<tr>
<td>SECONDARY CASES</td>
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<td></td>
</tr>
<tr>
<td>(1) Direct movement from an I.P.</td>
<td>146</td>
<td>12.9</td>
</tr>
<tr>
<td>(2) Movement from I.P. via a Market</td>
<td>104</td>
<td>9.2</td>
</tr>
<tr>
<td>(3) Market contact infection</td>
<td>107</td>
<td>9.4</td>
</tr>
<tr>
<td>(4) Vehicle contact infection</td>
<td>21</td>
<td>1.8</td>
</tr>
<tr>
<td>(5) Breeding movement infection</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>(6) Carrier Sow syndrome</td>
<td>32</td>
<td>2.8</td>
</tr>
<tr>
<td>(7) Indirect contact (via persons and/or things)</td>
<td>95</td>
<td>8.4</td>
</tr>
<tr>
<td>(8) Local spread</td>
<td>63</td>
<td>5.5</td>
</tr>
<tr>
<td>(9) Persistence of infection from a pre-eradication outbreak</td>
<td>5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Figure 7

TRACING OF CONTACTS

Direct pig to pig contact has always been recognised as the most likely method by which the disease is spread. There is now, however, a quite considerable weight of circumstantial evidence to show that the disease can be quite readily spread by indirect means, particularly by human agency and possibly by ground vermin acting as mechanical carriers. In the early stages of the eradication programme it was routine practice to place under restrictions premises contiguous to the infected place and to carry out regular veterinary inspection. These herds often became infected for no obvious reason and in consequence it has become routine procedure to slaughter as dangerous contacts any pigs which appear to be seriously exposed to infection by reason of contiguity to the infected place. Similarly if swine fever infects one of several herds in the same ownership, with common services for example attendants and feedingstuffs, it is unlikely that the other herd will escape infection and it has become usual to slaughter these herds as dangerous contacts.
All pigs which have been moved from the infected place at a time when infection is judged to have been present, are traced and if found healthy restrictions are served on the herd to which they have been moved until the danger period is likely to have elapsed. Originally the period of restriction was 28 days following the last date of exposure to infection but it has been noted on a number of occasions that although no marked illness occurred in the contact pigs and none of them died, illness and death from swine fever did develop in other pigs on the premises to which they were moved, and when the herd was slaughtered, well established lesions, characteristic of swine fever, were found in the primary contacts. In consequence of this it is now routine practice, in order to allow time for the disease to spread to susceptible contacts in the herd, to impose restrictions for 42 days on herds to which pigs which are at risk have been moved.

If contact pigs can be traced before they are likely to have become infective, they are slaughtered as dangerous contacts in the hope of saving the herd to which they have been moved. Usually this type of contact comes into one of the following categories:—

1. Pigs which were moved from an infected place within six days preceding confirmation of the disease and which have been immediately traced and found to be healthy.
2. Pregnant females which have been moved from the infected place during any time when infection is judged to have existed there providing that, when traced they are still pregnant and still apparently healthy.

Store pig markets are often involved in the spread of swine fever. In 1963-64, 36.5 percent of all the secondary cases were caused by infection introduced by market pigs and in 1964-65 the corresponding proportion was 56.5 percent. When the disease is confirmed in a herd and pigs have been sent to market at a time when it is judged that infection could have been present in the herd, the tracing of these pigs and of all market contacts is regarded as a matter of urgency. Similarly when it appears that swine fever has been introduced to the herd by pigs purchased from a market the tracing and restriction of all other pigs which have passed through the market on the same day is regarded as an urgent necessity.

INFECTED AREAS

The effective control of movement of susceptible animals is essential in any disease eradication programme. In Great Britain it is possible to operate a system of movement control by means of "Infected Area Restrictions" whenever the disease situation requires it.

When an infected area is declared all movement and marketing of pigs is prohibited in that area, except that licences may be issued for the movement of pigs for slaughter or direct from one farm to another. The imposition of these infected area restrictions has been used freely during
the eradication campaign with good results, especially in circumstances where infective pigs have passed through a market.

VACCINATION

When the eradication policy was introduced crystal violet vaccine was being widely used throughout the country, its use being greatest in those areas with the highest incidence of swine fever. Because the disease was then widespread, it was decided that immediate withdrawal of vaccine would not be justified, but in August, 1964, the supply of vaccine was discontinued for the following reasons:—

1. There had been a considerable reduction in the incidence of swine fever;
2. Experience had shown that vaccination was liable to hinder the recognition and elimination of infected herds with consequent danger of spread to other herds;
3. There had been a considerable reduction in the demand for vaccine.

Since the vaccine was withdrawn there has been no serious upsurge of the disease in any area.

PROCEDURE ON THE INFECTED PLACE

After confirmation of swine fever, valuation and slaughter of the affected herd is carried out with the least possible delay.

Because blood is the medium in which virus is most likely to persist on the premises, it is the practice whenever possible not to slaughter pigs in their individual pens but to move them to one slaughter area on the premises. In addition to limiting the area on which blood is shed and which therefore requires particularly careful disinfection, this has the added advantage of making the handling of carcases an easier task.

Because of the danger of perpetuating the swill hazard, no attempt is made to salvage the carcases for food. Within the limits of this restriction, the choice of method of carcase disposal in any outbreak rests with the divisional veterinary officer in the field. The choice will usually be determined by the number of carcases involved, the availability of suitable processing plant, the type of ground involved, etc. Three methods of disposal of carcases are in common use:—

1. The use of carcase processing plant, which is favoured when carcase numbers are not too great. Before it is approved for this purpose the plant is inspected by a veterinary officer to ensure that the type, location and layout are suitable and that the operators are prepared to adopt the stringent conditions of hygiene and disease security which are required. Carcases are always moved from the infected place in covered, drip-proof trucks some of which are equipped with lifting gear to facilitate loading and unloading. The whole operation of disposal and disinfection at the
processing plant is supervised by a technician on the staff of the divisional veterinary officer.

2. Burial on the premises is the next method of choice. The use of mechanical earth moving equipment makes this a quick method of disposal for large numbers of carcases.

3. Cremation of carcases on the premises is usually employed only when other methods of disposal are impractical.

Current rations of feedingstuffs are destroyed and apart from the area where blood has been shed disinfection of the premises is not an elaborate procedure. It consists mainly of soaking the whole premises by spraying with an approved disinfectant and the restrictions are usually removed 14 days after completion of this disinfection. There has been no case in which recurrence of the disease after restocking is believed to have resulted from the previous infection on the premises.

CONCLUSIONS

The progress towards complete eradication has proceeded in a satisfactory manner and the main difficulty encountered has been the problems of sub-clinical infection in pregnant sows. During the two years' work of eradication, however, a progressive and considerable reduction has been achieved in the national weight of infection and in the extent of distribution of the disease. The swill hazard has also been overcome and although no forecast is attempted as to when final eradication will be reached, it is confidently believed that if all concerned with pig production are vigilant and alive to the need to notify suspicion of disease without delay, complete eradication can be achieved.

SUMMARY

The paper presents an account of two years' work of swine fever eradication in Great Britain, which was begun on 11th March, 1963. The method of dealing with herds which were known to be infected on that day is described; the national incidence and distribution of the disease during the two years is outlined.

Diagnosis presented few difficulties in the early stages, but later the disease being encountered has been of a milder type and laboratory aid has been employed more frequently. The problem of transplacental infection in pregnant sows is a serious complication and congenital tremor in baby pigs is one of the defects associated with the exposure of their dams to swine fever virus.

The discovery of sources of infection is regarded as an essential step towards final eradication, and it is pointed out that as the programme advanced there was a marked increase in the percentage of cases in which a precise source of infection was established. There was also an encouraging reduction in the number of cases thought to be caused by infection from swill.
### SWINE FEVER

#### Tracing of all movements is carried out in great detail. In addition to the movement of pigs, indirect contact is regarded as important in the spread of infection. Where contact pigs can be traced before they are likely to have become infective, they are slaughtered with the object of saving the herd to which they have been moved.

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of outbreaks</th>
<th>Number of pigs slaughtered</th>
<th>Average number of pigs per case</th>
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<tr>
<td><strong>1963</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11th-31st March</td>
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<td>179</td>
<td>33,076</td>
<td>184</td>
</tr>
<tr>
<td>May</td>
<td>136</td>
<td>36,623</td>
<td>269</td>
</tr>
<tr>
<td>June</td>
<td>100</td>
<td>21,289</td>
<td>213</td>
</tr>
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<tr>
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<td>31</td>
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<td>277</td>
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<td>254,786</td>
<td>221</td>
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<tr>
<td><strong>1964</strong></td>
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<td><strong>1965</strong></td>
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<tr>
<td>January</td>
<td>17</td>
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<td>407</td>
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<tr>
<td>March</td>
<td>21</td>
<td>4,150*</td>
<td>198</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td>58</td>
<td>17,823*</td>
<td>307</td>
</tr>
</tbody>
</table>

Total compensation paid in financial year ended 31.3.63 £324,740
Total compensation paid in financial year ended 31.3.64 £3,630,992
Total compensation paid in financial year ended 31.3.65 £1,280,820*
Compensation paid or payable in 1965/66 financial year £14,000*

*Provisional figures
The prohibition of movement and marketing of pigs in a district is achieved by declaring the district to be an "Infected Area."

Vaccination is believed to hinder the recognition of herds which have become infected and vaccine was withdrawn in August, 1964.

Care is taken to avoid the spilling of infected blood over a wide area at the time of slaughter of infected herds. No salvage for food is carried out. Carcases are disposed of in processing plants or by burial or cremation.

Disinfection other than of the area where blood has been shed is not elaborate. Infected farms are freed after 14 days.

The opinion is expressed that eventual eradication can be achieved.

REFERENCES


* * * * * * * *

In introducing this paper the author said:—

You will know that I use the term "swine fever" to describe the disease which you refer to as "hog cholera." I am sure you will be tolerant and understanding of my continued use of the term which for me is customary usage.

(In the working paper which I submitted, there is a factual account of the methods we have used, and the progress we have made, during the first two years of our work of eradication.) I believe I can best employ the time you now afford me, by giving a quick account of our progress during the most recent six months, then describing in some greater detail the veterinary problems we have encountered in the field.

During the 10 years preceding the introduction of eradication, the average national incidence had been about 1,200 outbreaks per annum. At that time we regarded swine fever as being essentially a disease of feeding pigs, characterised by severe clinical illness which was associated with a high morbidity and mortality in herds which it attacked. We believe these 1,200 cases each year were an accurate representation of the number of cases of this type of swine fever which occurred in the country, but we have now so revised our ideas on swine fever as it occurs in Great Britain, that we do not now believe, that they were a true indication of all the swine fever which was occurring at that time.

It is nonetheless true, that the disease even as we recognised it, represented a serious loss to the pig industry and for some years opinion had been growing, that if eradication by stamping-out was a practical possibility, we could not afford to continue to live with a disease which took such a heavy toll of our pig herds each year.
The success of eradication in Canada, in Australia, in Northern Ireland and in the Republic of Ireland, encouraged us to believe in the practicability of eradicating the disease by a stamping-out policy, although we were well aware at the outset, that our task was likely to be much more difficult than theirs, since with us the disease had reached a well-entrenched enzootic state, which they had not experienced.

Figure 9 is a representation of the geographical distribution of outbreaks during the fifth six-months period (which ended on 30 September 1965). It shows the continued freedom of the large areas of the country which had been free from the disease during the previous six months, and that in addition further areas in South East England and in the Midlands of England also remained free from the disease.

In many of the counties where the disease was recorded during this period, e.g., Devon, Wiltshire, Lindsey, Flintshire and Denbighshire only one outbreak was confirmed.

Of the 47 cases which were confirmed during this period, 12 only occurred in the last three months, nine in July, one in August and two in September.

When we look critically at our two and a half years' work, we think that there is some justification for mild satisfaction in what we have achieved thus far, and for quiet confidence in the future. We are, however, anxious to guard against the danger of complacency and we are ever mindful of the sobering realisation which has been forced upon us, namely that when we undertook this task, we did so with a very limited understanding both of the nature of the disease itself and of its epidemiology in our country. We feel that we now know enough about both these subjects, to convince us at least that we do not know everything.

There are many problems associated with the eradication of this disease, but pre-eminent among those, in my view, is the essential veterinary problem of diagnosis. I feel sure that no veterinarian who has ever been charged with the responsibility for a swine fever eradication programme could long escape this conclusion.

In my own experience, having arrived at this conclusion, the next follows naturally from the first, and it is that in addition to adequately equipped and staffed laboratories where modern diagnostic procedures can be carried out, the programme requires a field veterinary staff, well informed and kept up to date on the clinical symptoms, morbid anatomy and epidemiology of the disease; also on the procedures necessary for diagnosis.

In these days of highly refined laboratory techniques as an aid to the diagnosis of swine fever, there is sometimes a tendency to minimise the importance of the clinician. This is not a view to which I subscribe. There is, I believe, no substitute for the experienced, observant clinician, but in a swine fever eradication campaign the clinician must be acclimatised to an acceptance of the truth, that while certain clinical symptoms and certain post mortem changes are fairly regularly encountered in swine fever, none of these is of itself diagnostic of the disease, nor is its absence conclusive evidence of the non-existence of the disease.
Swine Fever Eradication - Great Britain
Incidence and Distribution of Outbreaks: 1st April - 30th Sept, 1965

Figure 9
The most authoritative clinician on this disease is the one who by his own observation and experience, has become convinced that only by employing the fullest range of laboratory aids to reinforce his own observations and his knowledge of the case, can he be sure of reaching an accurate diagnosis of swine fever or, what is often more important in the context of an eradication programme, an accurate diagnosis of the non-existence of the disease.

In addition to the mild clinical form of the disease in feeding pigs which came to light during the programme, another and even more complex feature of the disease, was presented by trans-placental infection in the apparently healthy sow or gilt.

I understand that until recently this has not been recognised as a feature of the hog cholera problem in the United States. In Great Britain, before eradication, the fact had been recognised that breeding sows which survived an outbreak of swine fever were liable to give rise to trouble later, but, neither the true nature of the problem, nor its full significance in the epidemiology of the disease, were realised.

I believe, that in the custom of marketing pregnant animals, which is common in Great Britain, may lie the factor which creates the importance of this problem for us. I understand that such a practice is not commonly followed in this country, and while this may well explain why the problem should be of less significance here, I have always found it difficult to understand its apparent absence as a feature of the hog cholera problem in this country. I think, however, it may interest you if I describe the problem which emerged in our work of eradication.

The problem came to the forefront in the fifth month of the programme, by the disclosure of the disease in a breeding herd from which 16 pregnant animals had been sold to six different herds some two months previously. In all of these six herds the purchased sows remained perfectly healthy, except that they produced defective litters, and the existence of swine fever was confirmed either in the survivors of these litters, or in other pigs on the premises.

From enquiries which we have pursued since, we find that there is evidence of trans-placental infection being a feature of nine percent of all cases which occurred in 1963-64 and of 17 percent of cases which occurred in 1964-65. It is not therefore to be regarded as an insurmountable problem of eradication—once the existence of the problem has been recognised.

When trans-placental infection occurs in a herd the cases do not follow an identical pattern; we have found however that in broad terms, three main patterns emerge:—

1. The apparently healthy sow farrows a litter of piglets, all of which are lost by stillbirth or neo-natal death. Following this occurrence, symptoms of swine fever develop in other pigs on the premises.

2. The apparently healthy sow farrows a litter, some of which are lost by stillbirth or neo-natal death, and symptoms of swine fever
develop in the survivors of the litter at about the third week of life.

3. The apparently healthy sow farrows a litter of live pigs, some or all of which, are affected with congenital tremor.

It has been noted that such variations as dead, mummified, live trembling and live normal piglets may be present in one litter.

We have been unable to relate a pattern of foetal abnormality to the stage of pregnancy at which infection of the dam occurs, but in the evidence we have accumulated there is a suggestion that, congenital tremor does not occur in baby pigs as a feature of swine fever unless exposure of the dam to virus began in the early stage of the pregnancy.

In many of the cases in which congenital tremor was the only clinical symptom, the existence of swine fever was confirmed and the herd slaughtered before the opportunity for spread occurred. However, in the history of several cases which were confirmed because of the existence of swine fever in feeding pigs, there is evidence to suggest that the disease had spread to these pigs from a litter affected with congenital tremor which had been born some weeks previously, and we think that if swine fever virus is giving rise to congenital tremor in baby pigs, and the herd is allowed to survive, symptoms of swine fever, which may be mild symptoms, will eventually develop in other pigs in the herd.

I must stress here that we do not believe that all cases of congenital tremor in baby pigs are associated with swine fever virus. Indeed, our records show that of the cases which we have examined which showed this symptom, only 10 percent were attributable to swine fever.

I am sometimes asked what things I would wish to do differently if we were starting on our eradication programme now with the advantage of the experience we have gained.

There are three points to which I would give particular attention:—

1. I would in the beginning arrange a wide campaign of advisory lectures and demonstrations, both for veterinary officers within the Department and for general practitioners, to acquaint them with the true complex picture of swine fever in the field, both as an acute killing disease and a mild illness of feeding pigs, and also as a complication of pregnancy in breeding sows. I would emphasize also that this is a highly contagious disease in which transmission by mediate as well as immediate contact readily occurs.

2. I would seek legislation to control the sale and marketing of pregnant sows and gilts.

3. I would investigate the economics of providing centres where apparently healthy contacts could be slaughtered, processed, and canned for human food, and inedible material processed for fertilizers.

We know, and we have made this clear recently in our national publicity, that the apparently favourable position in which we now find ourselves, is a reality only if every illness of pigs which might be swine
fever, is being referred to us for investigation. This is an aspect of the problem for which responsibility rests with the veterinary practitioner and the farmer.

After two and a half years of our policy of eradication, we are determined that such success as we have achieved must not be endangered, for it has not been easily or inexpensively won.

In terms of human endeavour, there is no readily available yardstick by which to measure the expense, but this in general terms I can say with full knowledge; to be successful, a policy of eradication by stamping-out has to make tremendous demands upon a veterinary staff, requiring of them a painstaking and sustained application in the field to a duty which is at best uncongenial and often frankly unpleasant.

In terms of financial expense, the cost so far to the nation, in compensation alone, has been over £5-1/2 million, which is the equivalent of $15 million.

We believe that we have reached a stage in our programme where we can now say with confidence, that, if all concerned with the pig industry play their part, then final eradication from Great Britain, of this disease which we call "swine fever" will be achieved.
STATUS OF STATE-FEDERAL HOG CHOLERA
ERADICATION PROGRAM

G. H. Wise, D.V.M.*

The campaign to eradicate hog cholera appears to have progressed through two broad areas of development. The first of these took place from the early 1950's to the early 1960's and was concerned with evaluating probability of success and resources needed for the program. The second involved organization of program guidelines, establishment of procedures, and implementation of control phases in the field. This started with the annual meeting of this Association in Washington in 1962 and now is generally operative in most parts of the country.

We now face the final—and difficult—task of moving from control into actual eradication measures. Progress to date provides a valid basis for predicting final success—but such a prediction must be conditioned with the realization that complete eradication will not be achieved without more intensive effort than has been required up to this point.

GENERAL PROGRAM STATUS

Forty-nine States and Puerto Rico are engaged in the cooperative program. During the past year there has been continued progress in moving toward advanced phases of the program, with forty-five States now past the initial stage—including five in Phase III and nine in Phase IV. With the addition of Nevada in September, two of these nine have been established as hog cholera free. The far Western States now form a block operating at final eradication levels. The North Central and Northeastern States are generally in Phase II, with the Southeast and Southwest having States at various levels of progress.

During regional meetings held in the Corn Belt in July it was brought out that most States in this area expect to enter final eradication phases during 1966.

INCIDENCE OF HOG CHOLERA

More reports of illness suspicious of hog cholera were received in fiscal year 1965 than in fiscal year 1964—1727 as opposed to 1597. This is a desirable situation. In this report last year mention was made of the need to keep suspicious reports coming in, and the vital

*Senior Staff Veterinarian, Swine Diseases Section, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
importance of constant stimulation of all those concerned with hog cholera eradication to maintain a high level of reporting.

The contrast between suspicious reports and the number confirmed as hog cholera is a prime tool in evaluating our situation. During 1965 confirmed reports were slightly below 1964—1110 as opposed to 1118. Another way of expressing this is that 64 percent of the suspicious reports were confirmed in 1965, a drop from 70 percent during 1964. The spread between suspicious reports and confirmed cases is still too narrow. Constant attention to maintaining reporting of suspicious illness at the highest possible level is necessary—particularly in view of the increasing recognition of less obvious forms of hog cholera which would have been largely disregarded only a few years ago.

Two items of significance should be mentioned in connection with incidence in 1965. One is that a disproportionate share of confirmed cases occurred in the Southeast—primarily in three States (Georgia, North Carolina, and South Carolina) which accounted for 46 percent of all confirmed cases in the country. Intensive effort in Georgia, the State faced with the biggest problem, was successful in containing the disease and reducing incidence. During May 103 outbreaks were reported from that State, with 52 during June. In July and August this
Cooperative State-Federal
Hog Cholera Eradication Program
October 1, 1965

Figure 2

Hog Cholera Eradication
OUTBREAKS REPORTED

Figure 3
dropped to 17 cases for each month. Thirteen cases were reported in September. We all should learn from their experience, particularly in gaining an appreciation of the amount of manpower and time required to adequately cope with an outbreak that builds rapidly in a concentrated swine producing area.

Whether or not there is a contributing environmental factor in this part of the country is not clear. This may be deserving of study, although Florida, with similar environmental and husbandry conditions, was successful in stamping out the two outbreaks reported there in 1965, with no evidence of further spread.

The other area of major interest regarding incidence is in the Corn Belt, where confirmed outbreaks in 1965 dropped 35 percent from 1964. As these States have the bulk of the swine population, this is particularly significant.

There are two swine inspection activities, other than those in the field program, which are of interest in evaluating apparent incidence. These are the inspection of swine at slaughter by veterinarians of the Meat Inspection Division and the inspection of swine by veterinarians at public stockyards. Both activities preceded the hog cholera eradication program. A comparison of their work as regards animals suspicious of hog cholera can be made for years prior to and during the

![INDEXES OF HOG CHOLERA INCIDENCE](image)

**Figure 4**
program. Both report a drop in the rate of suspicious hog cholera found in the years since the program was initiated—Meat Inspection Division condemnations have dropped from about 0.5 per 10,000 carcasses inspected in the years preceding the program to less than 0.1 in 1965. Public stockyard veterinarians report a drop in suspicious cases from about 0.4 per 10,000 swine inspected in prior years to less than 0.1 in fiscal year 1965.

**DIAGNOSIS**

There has been continued improvement in the use of the laboratory for diagnostic support. Prior to the program it was reported that laboratory assistance was sought for about one-third of the cases. In 1964 this rose to 60 percent, and during the past year to 80 percent. A goal of 100 percent laboratory assistance is necessary, and the laboratory studies must include all procedures available to give maximum information. Diagnostic guidelines adopted in 1964 by this Association and the Animal Health Division call for a combination of field and laboratory data before a determination is made. This procedure
was the result of a two-year study by USLSA, the Conference of Veterinary Laboratory Diagnosticians, and the Division. All laboratory procedures included in the guidelines are available in all sections of the country, either through the State laboratories or through the National Animal Disease Laboratory. At this stage of program effort no final determination of a suspicious hog cholera case should be considered complete unless the full scope of information from the laboratory is available to the field veterinarian in making the decision.

In this country, as in other parts of the world, we are becoming increasingly aware of the less obvious clinical cases of hog cholera, as well as the potential importance of the carrier animal as a means of transmitting virus to susceptible contacts. Examples of these were reported from several parts of the country during the past year.

In Tennessee a low-grade illness persisted for several months in an unvaccinated herd. Improvement seemed to take place following medication, but the sickness would recur following cessation of treatment. Hog cholera was eventually confirmed in the herd. In South Carolina two cases were reported in which hog cholera recurred several months after the original outbreak in the herd had apparently subsided and quarantines had been removed. Also in South Carolina, where all swine tissues coming to the laboratory are examined for hog cholera, positive results for hog cholera have been picked up on some cases where there had been no suspicion in the field that this was involved in the trouble.

In Indiana, a study of obscure illness in suckling pigs from unvaccinated sows also resulted in positive laboratory results. The sows had been in contact with freshly vaccinated pigs during pregnancy.

These are illustrations of the necessity to utilize all available aids before concluding that hog cholera is not present.

**SOURCES**

Apparent sources of hog cholera outbreaks this past year were reported from 61 percent of the completed investigations, as compared with 55 percent in 1964. Outbreaks from intrastate movement increased, while those from interstate movement decreased. Our controls over swine movement, to prevent spread of hog cholera, still need improvement. Spread within local areas also showed an increase. Reducing this means of spread is dependent upon prompt effective quarantine measures and properly informed swine owners. We need to improve here also. Vaccine caused outbreaks were reported less frequently in 1965 than in 1964. Outbreaks attributed to the feeding of raw garbage rose slightly during the year.

Field investigations by State or Federal veterinarians were reported for 98 percent of the suspicious reports received in 1965. This, coupled with some increase in success in tracing apparent sources, demonstrates progress. With this aspect of the work, as with the use of the laboratory, the aim must be not only for coverage of all cases, but
also for as thorough study as possible. Conclusions drawn should be based on the evidence available after all sources of information have been explored.

VACCINATION

Data furnished by the Veterinary Biologics Division indicate that sales of hog cholera vaccines increased in 1965, rising about 3.5 million doses over 1964. During the same period there was an estimated drop in swine production of about eight percent as compared with the previous year. Hog cholera outbreaks attributed to vaccine use also decreased slightly in 1965, but still accounted for 13 percent of all outbreaks for which a source was reported. Analysis of field reports from vaccine caused outbreaks continues to show that in most cases the products were used other than as recommended by the manufacturer. There are also indications that some products may have a higher degree of safety under field conditions than others. At this stage of the eradication program vaccine safety is of increasing importance. Present safety standards are largely those that were adopted at the time hog cholera immunization was moving from virulent virus to the attenuated products and before the eradication program was initiated. The
Veterinary Biologics Division, in view of these changing circumstances, has under study the question of revised safety standards.

COMMENTS

Program data continues to indicate that the basic requirements for hog cholera eradication, adopted by this Association at the beginning of the campaign, are sound and effective when applied with thoroughness and understanding.

The importance of prompt reporting of suspicious illness in swine, comprehensive diagnostic procedures and epidemiology, and effective inspection and quarantine activities cannot be underestimated. Efforts in these areas must be intensified in the years ahead. The increasing evidence, from this country and abroad, of the potential for unrecognized virus carriers to continue the spread of infection illustrates the necessity of a full "stamping-out" program in order to achieve final eradication. Such evidence reinforces previous recommendations of this Association on the need for herd depopulation in final phases of the program and for constant survey of the swine population in areas considered free of the disease. Anything less is control rather than eradication. Livestock sanitary officials must be the leaders in emphasizing the necessity of such measures in discussions with others working to achieve the goal of the program.
REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA


Since the last report of this Committee to this body we have been pleased to note varying degrees of progress in the cooperative hog cholera control and eradication programs, and the vigor with which the program has been pursued in some of the states. All states save one are now engaged in the national effort. Two states have achieved the Hog Cholera Free status and seven others are in Phase IV. It is significant to point out that the states in the Cornbelt, where the majority of our swine is located, have made great strides in reducing the incidence of the disease and are nearing the stage where an all-out eradication effort may be instituted.

In view of the goals previously considered by this group which call for practical eradication of hog cholera by the end of 1969, the Committee re-emphasizes the urgency for all states which have not done so to secure the necessary legal authorization and funds for indemnification, if contemplated, in the implementation of the final phases of the eradication program.

Consideration has been given by your Committee to the movement of swine between states, and it is felt that some recognition should be shown those states which have attained the status of free areas and eradication areas. The Committee therefore endorses the proposed amendment to the code of federal regulations which would permit the movement of swine from such free areas and eradication areas without the necessity of vaccination against hog cholera, subject to the individual requirements of the states of destination, and recommends approval of this action by this Association.

The Committee has considered proposals which emanated from workshop meetings in the Cornbelt area sponsored by Livestock Conservation, Inc., and the Agricultural Extension Service, aimed toward obtaining uniformity in the sanitary standards and facilities in swine marketing centers. Your Committee wishes to recommend that cooperating state and federal authorities in the several states, along with market operators and members of the swine industry, make a survey of the state-federal approved markets to determine if the standards established in 1962 are being carried out, and to provide a basis for evaluating the adequacy of these standards. A report of the survey should be made to the Association.
through this Committee at its next meeting, along with recommendations necessary for establishing adequate standards.

In addition, the suggestion came from these workshops that feeder pigs moving in commerce should be limited to passing through one market before going to the farm of final destination. There exists some doubt as to whether or not this suggestion involves interstate commerce only, or both intra and interstate commerce. It is also unclear as to just where the one leaves off and the other begins, and certainly the area of legal responsibility and authority are anything but clearly defined and understood. Your Committee feels that these things should be made perfectly clear and that whatever legal assistance and counsel is necessary be secured by the United States Department of Agriculture in studying this problem so that a clear-cut recommendation may be made next year.

The Committee received a report from personnel of the United States Department of Agriculture Veterinary Biologics Division, National Animal Disease Laboratory, concerning methods which have been developed at that facility for the titration of anti-hog cholera serum and the identification of completely susceptible pigs. These findings will permit the evaluation of serum on the basis of relative potency, provide for the selection of the most suitable pigs for test purposes, and contribute to the development of additional vaccine safety and serum potency tests. The Committee is pleased to note that the Veterinary Biologists Division is improving its methods for analyzing these anti-hog cholera products so vital to the eradication effort. We confidently expect that this work will go forward with vigor.

In view of the data presented on this preliminary work being conducted to determine the spreading potential of some modified live virus vaccines, your Committee recommends that a critical review of our licensed vaccines be undertaken with the goal of eliminating those on the market which are capable of spreading clinical hog cholera.

PROPOSED REVISION OF THE 1965 REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA

This revision and substitute for the last paragraph of the report was sent to each member of the Executive Committee and received 39 votes in favor and 4 against the proposal.

Delete the last paragraph of the 1965 Committee Report and substitute the following:

Your committee recommends that the provisions of Phase III be revised as follows:

Phase III - Elimination of Outbreaks

A. All steps included under Phase I and Phase II have been and are being carried out effectively.
B. Depopulation Plans for Infected Herds. Such plans shall meet the following minimum requirements:

(a) Prompt disposition of all swine in the infected herd. Marketable, apparently healthy swine from such herds may be salvaged for slaughter, without special processing, subject to the following:

(1) A State or Federal veterinarian shall determine the swine that are marketable immediately prior to loading. Such determination will be based on clinical examination, including temperature observation as necessary.

(2) The swine shall be accompanied by a permit issued by the appropriate official and shall move in a sealed vehicle directly to a designated establishment for slaughter.

(3) All such vehicles shall be cleaned and disinfected under supervision following unloading and before again being used.

C. All other swine in the infected herd to be condemned and destroyed, with provision for indemnification as necessary to accompany depopulation. Preliminary classification for final appraisal purposes should be made at the time of the initial visit to the suspected premises by the regulatory veterinarian.

(a) Disposal of condemned swine will be under the supervision of a regulatory employee. This may be by complete burning on the premise, deep burial, or by hauling direct to a dead-animal disposal plant in a leak-proof, covered, sealed vehicle. Such vehicles shall be cleaned and disinfected under regulatory supervision immediately following unloading.

(b) Nonprocessed salvage of apparently healthy marketable swine as provided herein represents a minimum provision for Phase III operation and will be discontinued in favor of total herd disposition without such salvage in any case or at any time when it is determined by cooperating officials that the procedure would be impractical or otherwise constitute an undue threat to the eradication program.
Most authorities in the field of disease control and eradication will agree that it is not difficult to markedly reduce the incidence of a given disease once interest and efforts are mobilized to accomplish this end. Most authorities will also agree that marked reduction in the incidence of a given disease and complete eradication of this given disease are two entirely different concepts. In fact, they agree that the problems of complete eradication really begin when the incidence is reduced to a small fraction of its original intensity. We are at this point today in the eradication of bovine tuberculosis.

This presentation does not contain any startling new information regarding the eradication of bovine tuberculosis. Rather, it is a report of the circumstances associated with an outbreak of bovine tuberculosis in Tennessee. It attempts to focus attention on some of the problems associated with tracing lesion cattle found on regular kill. In the interest of clarity, this presentation will be given in the form of a narrative case report.

In December 1963, a bull being slaughtered at a federally inspected slaughterhouse in Nashville, Tennessee, was retained because of a slight, peculiar appearing lesion in the thoracic cavity. Closer examination of the animal revealed this to be the only gross lesion present. The lesion itself was slight and very atypical of bovine tuberculosis. It was located on, rather than in, one of the posterior mediastinal lymph glands. The appearance of the lesion was that of a clump of small spherical nodules located adjacent to the gland. Three or four of the nodules were attached to the gland, but the remainder were positioned away from the gland in the pleura and fat of the mediastinum. The nodules were three to five millimeters in diameter, very thin walled, and a golden yellow color. The contents of the nodules consisted of a soft, moist, caseous material.

Four veterinarians, all having experience with bovine tuberculosis, examined the lesion grossly. Due to its unusual location and appearance, all those present were of the opinion that the lesion was not due to infection with *Mycobacterium bovis*. It was decided, however, to submit specimens to the National Animal Disease Laboratory for histopathological and bacteriological examination. The usual ADE Form 6-35 was accomplished and submitted.

Immediate steps were taken to determine the origin of the animal. The hide revealed that the animal was solid black; the conformation and weight showed that it was of beef type; the left ear bore a partially legible tattoo that appeared to be either 648 or A4P; and the dressed weight of the carcass was 682 lbs. Plant records showed that the bull
was from a lot of only five animals. All five animals were bulls and had been purchased the previous day through the Nashville Union Stockyards. Two of the five bulls were black and of the Aberdeen Angus breed. Both had been purchased from the same commission company and had gross weights of approximately 1250 lbs. and 1450 lbs., respectively. By applying normal dressing percentages, it was determined that the smaller bull was the one from which the lesion had been recovered.

The commission company readily furnished the names of the two men for whom they had sold the two black bulls. Since the lesion was so slight and atypical, it was decided to wait for the histopathology report from the laboratory before proceeding further with the investigation. The laboratory report, received during January 1964, indicated findings compatible with a diagnosis of tuberculosis. The owner of the small bull, a gentleman whom we will call John Doe, was informed of the situation. He produced registration papers on the bull he had sold which showed the ear tattoo to be 7H8. Considering the poor legibility of the tattoo, the Doe bull was still considered to be the one from which the lesion had been recovered.

Because of calving, and unseasonable rainy weather that isolated the herd from barns and holding facilities, testing of the herd was delayed approximately two months. During this time, the laboratory reported the isolation of *M. bovis* from the specimen. Mr. Doe requested that his private veterinarian be allowed to perform the testing. This was arranged and testing of the herd was accomplished during April 1964, using the caudal fold technique and a 0.2 ml. dose of tuberculin. The herd consisted of 29 good quality grade Aberdeen Angus cows. The entire herd was negative.

The tattoo discrepancy and the possibility of error in the computation of weights were considered and it was decided to test the herd from which the large black bull had originated. No registration papers were available on this animal. The herd consisted of 35 head of mixed breed cattle. The same testing technique was used. This herd was negative also.

Interest again centered around the origin of the small bull. It was traced back to the farm from which Mr. Doe had purchased him. This turned out to be a pure-bred breeder who had several hundred cattle. After overcoming several minor problems, including hot weather and a lack of eagerness on the part of the owner, arrangements were made to tuberculin test this group of cattle. During June 1964, 563 cattle in 10 herds were tested. All were negative to the tuberculin test. The record of the dam of the lesion bull revealed that she had been culled from the herd because of age in 1962. She had been sold through Nashville Union Stockyards and slaughtered in a federally inspected slaughterhouse. By this time, the investigation was beginning to appear hopeless.

During September 1964, the veterinarian who had tested the Doe herd was contacted about another matter and was informed of the
additional testing that had been performed in an effort to locate the disease source. He remarked that the source from which the bull had contracted the disease was anyone's guess since he was the biggest rogue in the county. When questioned as to the meaning of his statement, he replied that the bull spent more time in the neighbors' pastures than he did in that of Mr. Doe. Of course, this fact presented an entirely new set of circumstances.

Investigation revealed that there were five farmers who owned cattle and pastured them at some time during the year in pastures adjacent to those occupied by the Doe cattle. Due to the fall harvesting of crops and the fact that a portion of these herds were scattered on summer pastures, testing of these herds was delayed until late fall of last year. All five herds consisted of grade commercial beef cattle.

In November 1964, the five herds were tuberculin tested. Three of them were negative. In the fourth herd, which we will designate Herd A, 41 cattle were tested revealing 14 reactors. In the fifth herd, which we will designate Herd B, 43 cattle were tested revealing 21 reactors. Slaughter of the reactors from these two herds proved the infection to be extensive (Table I). Due to the overwhelming amount of infection in Herd B, the decision was made to indemnify the entire herd and destroy it. Post mortem examination of the non-reactors revealed two of the five remaining adult cattle to have tuberculous lesions. One was very extensive, to the point that the carcass was condemned. The other bore a slight lesion in one of the mesenteric glands. This increased the herd lesion rate to 88 percent in the adult age group. One approximately four-month-old calf, that was not tested, bore a well-marked lesion in one suprathyroid lymph gland.

In addition to the cattle, 12 breeding swine were tuberculin tested on Farm A and four on Farm B. One very old sow on Farm A reacted violently. This animal was quite thin and emaciated. The owner had been trying unsuccessfully for some time to fatten this animal to sell for slaughter. Autopsy revealed extensive tuberculous lesions of the liver.

After all doubt had been erased from the herd owners' minds as to whether or not bovine tuberculosis actually existed in their herds,

<table>
<thead>
<tr>
<th>Number Test</th>
<th>Number Reactors</th>
<th>Percent Reactors</th>
<th>Lesion Reactors</th>
<th>Condemned Rate No./Percent</th>
<th>Reactor Lesion Rate</th>
<th>Herd Lesion Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd A</td>
<td>41(A)</td>
<td>14</td>
<td>34</td>
<td>10</td>
<td>4/40</td>
<td>71</td>
</tr>
<tr>
<td>Herd B</td>
<td>25(A)</td>
<td>20</td>
<td>80</td>
<td>20</td>
<td>15/75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>18(Y)</td>
<td>1</td>
<td>5.5</td>
<td>1</td>
<td>-</td>
<td>5.5</td>
</tr>
<tr>
<td>Herd C</td>
<td>10(A)</td>
<td>10</td>
<td>100</td>
<td>9</td>
<td>4/44</td>
<td>90</td>
</tr>
</tbody>
</table>

A - over one year old.
Y - 6-12 months old.
the owner of Herd B disclosed that he owned 18 additional cattle on another farm, which we will designate as Herd C. Questioning revealed that they were actually a part of Herd B which, by now, had been slaughtered. The elderly father of the owner of Herd B claimed this group of cattle and pastured them separately during the summer until grass disappeared from the pastures in the fall. Herds B and C were brought together during the winter and maintained as one herd until the following spring.

In testing Herd C, the cervical technique was used. This herd consisted of 10 adult cows and eight small calves. All 18 animals were classified as reactors. Post mortem examination revealed no lesions in the calves; however, it showed tuberculosis to be extensive in the adult age group (Table I).

The owners of Herds A and B are the sons of the owner of Herd C. The cattle in all three herds originated from a herd formerly owned by the father. As a matter of fact, the father and two sons had been partners until 1953 or 1954 when the partnership had been dissolved and the cattle divided. The owner of Herd A had moved his cattle to another farm. The remaining animals were maintained together until around 1960, when the father started moving his cattle during the summer months. There was no significant history as to the origin of the cattle in the original herd. Very few cattle had been added to the herds since dissolution of the partnership.

Apparently John Doe's bull contracted the disease from Herd A. The two owners agreed that most of the bull's time away from home was spent with Herd A, and there was no connection between the bull and Herds B and C. Likewise, there was no direct connection between the cattle in Herd A and those in Herds B and C. All evidence indicates that tuberculosis existed in these cattle since prior to the dissolution of the partnership in 1953 or 1954.

A couple of interesting facts were uncovered to account for the difference in incidence rates between Herd A and Herds B and C. First, the owner of Herd A had acquired the youngest portion of the herd when the cattle were divided. Secondly, the owner of Herd A never confined his cattle inside a barn. They had access to an open barn during bad weather, but were allowed to go in and out at will. Not only did the owners of Herds B and C retain the older animals, but they made a practice of confining their cattle to a small barn during cold weather. The cattle, when confined, were quite crowded. This practice had been discontinued about 1961 because of respiratory difficulties thought to be pneumonia.

The cattle on 14 farms, either bordering the infected farms or having the possibility of exposure from them, were tuberculin tested. One 15-month-old steer on a farm adjoining Herd B was classified a reactor, but revealed no gross lesions at post mortem. All of the remainder were negative.

Herd A was retested during January 1965, using the cervical technique. Of 51 animals tested, 22 were classified as reactors. The
decision was made to indemnify and destroy the remainder of this herd. Of the 22 reactors, four adult animals had gross lesions, two of which were condemned. No gross lesions were found in the non-reacting animals.

From available records, 103 animals were traced from these herds. Most were feeder calves sold through organized feeder calf sales, that went to feedlots and subsequently to slaughter. Five of the 103 animals were purchased by three separate cattlemen. Individual identity of specific animals could not be determined, but all three herds were negative to the tuberculin test. To date, with the exception of the John Doe bull, nothing has been found to indicate that the disease has spread past the boundaries of the three farms.

The John Doe herd was retested in February 1965. All animals remained negative to the tuberculin test.

Historically, tuberculosis records for the county in which this case occurred show only one other herd in which the disease pattern resembles that of infection with *Mycobacterium bovis*. This was in a mixed dairy herd during the 1940's. Efforts to establish a connection between this herd and the three involved in this report have been fruitless.

The traceback case covered in this report spanned a period of approximately 15 months. It included countless hours of time and miles of travel, in addition to approximately 1200 individual tuberculin tests applied to 31 herds of cattle. Obviously, the element of fate or luck cannot be eliminated when considering the course of this traceback. Regardless, there are several features of this case that dramatically emphasize certain points that all persons associated with the tuberculosis eradication effort should keep in mind if the remaining foci of bovine tuberculosis are to be located and eliminated from the cattle population of this country (Figure I).

1. This case emphasized the value of tracing lesion cattle found on regular kill as a means of locating new infection. This is especially true in the case of commercial beef cattle under range or semi-range conditions. These herds had never or never would have been tested for routine reaccreditation.

2. It emphasizes the importance of animal identification in arriving at the correct farm of origin. Even though identification in this instance was not ideal, it was adequate.

3. It emphasizes the importance of submitting all lesions resembling tuberculosis, regardless of how slight or atypical, for laboratory examination. No one will ever know how close this case came to ending with the black bull back in December 1963.

4. The presence in one of these herds of two non-reactors with lesions of tuberculosis emphasizes the wisdom in destroying whole herds when the infection rate is high. As a matter of fact, tuberculosis eradication may be to the point where it would be wise to take this approach with all herds from which *M. bovis* is isolated.
SECONDARY HERD A

TESTED 41
REACTORS 14
LESIONS 10
CONDEMNED 4

SECONDARY HERD B

TESTED 43
REACTORS 21
LESIONS 21
CONDEMNED 15
N/R LESIONS* 2
N/T LESIONS** 1

SECONDARY HERD C

TESTED 18
REACTORS 18
LESIONS 9
CONDEMNED 4

* N/R non-reactors
** N/T not tested (4 mo. old calf)

Figure 1
5. The absence of reactors in the herd of origin of the lesion animal being traced emphasizes the importance of extending the traceback beyond the boundaries of the herd of origin. If there was a key to the case reported here, this would be it. In the event of an *M. bovis* isolation, every possibility should be exhausted. One can't help but wonder what the outcome would have been if the initial test of the John Doe herd had yielded four or five reactors with a few lesions.
PRESENTATION OF AWARD TO DOCTOR JOHNSON

F. J. Mulhern

_Speaker_: Dr. Francis Mulhern would like to make an award at this time. Doctor Mulhern.

_Dr. F. J. Mulhern_: Thank you, Doctor Safford. I think we can all appreciate the significance and importance of this traceback story that we heard, and the work that was done. It is an excellent example of how cooperation by a Veterinary Inspector paid off—because if it wasn't for the alertness on the part, and the participation of the meat inspector at the time of slaughter, this whole success story would not be told. The importance of the meat inspector, whether State or Federal, or municipal, who is alert enough in this area to recognize the responsibilities that he has as an individual to the State and to the country in finding the last vestiges of this disease, we think are highly important, to the point that we would like to give recognition to him by presenting a citation to this meat inspector who will represent other meat inspectors, whether in State, Federal, municipal inspection—for their total contribution to our effort. Doctor Johnson, of the Meat and Inspection Division, come forward please.

Gentlemen this is the meat inspection veterinarian that found this lesion case and reported it to us, and we are presenting this Certificate of Merit to him for his outstanding contribution to the State and Federal Cooperative Tuberculosis Eradication Program in providing the information which led to the identification of an unknown tuberculosis-infected herd. We wish to extend our sincere gratitude and appreciation for this fine work, to Doctor Johnson of the Meat Inspection Division.

_Doctor Johnson_: Thank you Doctor Mulhern for this Award.
THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

A. F. Ranney, D.V.M., M.S.*

Hyattsville, Maryland

A quarter of a century has passed since this country was declared Modified Accredited for bovine tuberculosis. The goal of eradication is still ahead of us. While progress toward eradication may not be as spectacular as we might wish, we should be encouraged by the progress that is being made.

The number of herds recorded with infection indicative of bovine tuberculosis is showing a steady decrease from 223 in 1962 to 141 last year.

The reasons for the tests that figured in the initial disclosure of infection in each of the 141 herds should be evaluated to determine the procedures that have proven effective in planning for the future of the program.

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**Tuberculosis Eradication**

**LOCATING INFECTED HERDS**

(Indicative of *M. bovis*)

Fiscal Year 1965

- Routine Testing 62%
  - Area 47%
  - Herd Accred 5%
  - Milk Ord. 4%
  - Sale-Show 4%
  - Import 2%
- Tracing 38%
  - Exposed 9%
  - Reactors 0.7%
  - Regular Kill (6-35) 29%

Total Herds - 141

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*Dr. A. F. Ranney, Chief Staff Veterinarian, Tuberculosis Eradication, Cattle Diseases, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Hyattsville, Maryland.*

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Here we show graphically the percentage results of the five reasons for routine testing and the percentage results of testing following the three tracing procedures (Figure 1). Of the 141 infected herds, 87 or 62 percent were originally detected as a result of routine testing. It is especially interesting to note that area testing and herd accreditation account for the finding of 52 percent of the herds with known bovine tuberculosis. The other 54 herds or 38 percent of the total were those disclosed following tracing procedures. (1) Herds to which exposed animals were moved, (2) Herds of origin of animals that reacted in another herd, (3) Herds of origin of animals with lesions reported on regular kill meat inspection.

The sooner that infection is detected in a herd the quicker eradication procedures may be instigated with a reduction in the chances of spreading infection (Figure 2). It will be observed that during each of the past two years considerably more herds with early infection were disclosed by routine testing than as a result of testing after tracing; also the contrast between early and advanced herd infection is significantly greater in the case of routine testing as compared to traceback.

The breakdown of the 141 herds into types of cattle involved is illustrated (Figure 3). Here it will be observed that 76 percent of the total were dairy herds while the remainder are shown to be beef animals or

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**STATUS OF DISEASE IN *M. bovis* INFECTED HERDS**

**FOUND BY: ROUTINE TESTING / TRACING**

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Total Herds</th>
<th>Routine Testing</th>
<th>Tracing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>184</td>
<td>74% (117 herds)</td>
<td>26% (67 herds)</td>
</tr>
<tr>
<td>1965</td>
<td>141</td>
<td>74% (87 herds)</td>
<td>26% (54 herds)</td>
</tr>
</tbody>
</table>

Figure 2
mixed dairy and beef (18 percent and six percent respectively). Of the 107 dairy herds, 80 or 75 percent were found as a result of routine testing. In the beef herds, 23 or 92 percent were found as a result of testing following tracing procedures. The mixed herds were about equally divided between routine testing and tests following traceback.

These data show that routine testing continues to be a major factor in detecting tuberculosis, especially in dairy cattle. This also supports the conclusion, drawn from data in prior years, that it is unsafe to abruptly curtail routine testing unless or until other procedures for promptly locating tuberculosis herds prove to be an adequate substitute.

The identification of cattle through accredited slaughter as a means of building up our traceback efficiency is dependent upon basic supporting actions such as:

1. Identification and history of individual cattle in the herd.
2. Adequate marketing records, including dealer records.
3. Simple, routine methods of maintaining animal identification through slaughter.
4. A consistent, organized reporting system for all cases of tuberculosis revealed on post-mortem examination.
5. Prompt epidemiological study of each case reported.
The movement of cattle to slaughter that show lesions of tuberculosis on regular kill, meat inspection, last year is shown (Figure 4). It may be observed that these cases involve movement from, through or into almost every state.

It has been pointed out in past reports to this Association that infected herds may appear almost any place in the country. Thus, we must be constantly alert for tuberculosis in herds of cattle and other animals that may contribute to the spread of the disease.

During Fiscal Year 1965, 115 additional counties were designated as areas accredited free of bovine tuberculosis in the domestic bovine (Figure 5). There is a total of 541 such areas in twenty States and Puerto Rico. One State, New Hampshire has an Accredited Tuberculosis Free Status.

The map shows the number of *M. bovis* infected herds in each State with the total for each of the four regions (Figure 6). Those States with five or more infected herds are shown in solid color. It is significant that all of the States in this category are among those where the officials are giving the tuberculosis eradication program special emphasis in their animal disease eradication efforts.

A low degree of bovine tuberculosis for the past quarter of a century should stimulate us to intensify our efforts to eliminate, from all animals in this country, bovine tubercle bacillus.

The number of Red Flag herds continues to decline. It will be
STATUS OF TUBERCULOSIS ERADICATION PROGRAM

Cooperative State - Federal
TUBERCULOSIS ERADICATION PROGRAM
July 1, 1965

[Map showing status of tuberculosis eradication program in the United States as of July 1, 1965, with states marked as eradicating or non-eradicating.

Figure 5

Tuberculosis Eradication
AGGRESSIVE PROGRAM NECESSARY TO FIND M. bovis HERDS

[Map showing distribution of tuberculosis cases by state, with 141 herds identified in fiscal year 1965.

Figure 6
observed that the number has been reduced from 239 to 14 in the past five years (Figure 7).

The number of tuberculin tests applied to cattle during last year was less than for any in the past 40 years.

There were 8.2 million tests reported in Fiscal Year 1964 compared to 7.1 million for Fiscal Year 1965. This is a reduction of 13.5 percent in tuberculin tests (Table I).

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis Eradication Comparative Results</td>
</tr>
<tr>
<td>F.Y.</td>
</tr>
<tr>
<td>1964</td>
</tr>
<tr>
<td>1965</td>
</tr>
<tr>
<td>Decrease</td>
</tr>
</tbody>
</table>

The number of reactors dropped from 8,225 to 5,608 or a decrease of 32 percent during the same period. This decrease in reactors is in part due to the lesser number of tests made. The percentage of cattle classed as reactors during the past fiscal year was 0.08 percent; in other words,
eight reactors per ten thousand animals tested, a record low.

If it were not for the attention being given to selective testing, we should view the marked reduction in testing with extreme concern. It is imperative that increased emphasis be placed on case studies associated with pathology of the disease reported by personnel conducting routine meat inspection examinations at accredited slaughtering establishments. Otherwise this will fail to be an efficient adjunct to the program.

TABLE II

<table>
<thead>
<tr>
<th>F.Y.</th>
<th>Reactors with lesions</th>
<th>Reactors with Generalized TB</th>
<th>Percent lesion reactors with generalized TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>1,557</td>
<td>253</td>
<td>16.2</td>
</tr>
<tr>
<td>1965</td>
<td>1,357</td>
<td>323</td>
<td>23.8 (all time high)</td>
</tr>
<tr>
<td></td>
<td>-200</td>
<td>+70</td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>12.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td></td>
<td></td>
<td>27%</td>
</tr>
</tbody>
</table>

A pertinent factor to be considered along with the decreased testing is that 16.25 percent of the lesion reactors slaughtered in Fiscal Year 1964 were reported with generalized tuberculosis as compared to 23.8 percent for 1965 (Table II). This noticeable increase in percentage of generalized cases (27 percent) resulted in part from increased traceback and better selective testing. There is also reason to believe that infection was present and allowed to spread for a longer period before being detected than for the prior year.

A study of the 141 herds with infection indicative of *M. bovis* revealed that 63 or 45 percent had been infected prior to Fiscal Year 1965. Of the 63 herds, 47 or 75 percent had been infected, quarantined and released from quarantine on one or more occasions during their infected history.

A change in United States Department of Agriculture regulations to permit the payment of increased indemnity for non-reacting cattle in certain problem herds is expected to stimulate the elimination of dangerous foci of infection. Twenty-two herds of cattle were eliminated last year with indemnity for non-reacting exposed animals. Some of these herds were infected for a prolonged period. The risk of further spread from these herds is now ended.

Laboratory examination of specimens suspected of being tuberculous is very valuable to the field veterinarian, both in assisting him in arriving at a herd diagnosis, and in deciding on the subsequent handling of the herd. Laboratory reports like other eradication tools, have their limitations. The field veterinarian must be aware of these limitations as well as the proper use of laboratory information. Information from the laboratory should be used as an aid to diagnosis giving due consideration to all epidemiological factors involved.
The cumulative data on 24 *M. bovis* herds in which specimens from 10 or more animals in each herd were submitted to the National Animal Disease Laboratory during Fiscal Year 1964 is summarized (Figure 8).

The following observations should be noted concerning the data on these 24 herds:

1. *M. bovis* bacteria were isolated from each of the 24 herds.
2. *M. bovis* was the only acid fast organism isolated in nine of these herds.
3. Multiple types of acid fast organisms were isolated in the remaining 15 herds.
4. *M. avium* was isolated from four herds. In two of them it was the only acid fast organism isolated other than *M. bovis*.
5. No isolation was made from some of the specimens submitted from each of these herds.
6. In none of the herds were all the histopathological examinations compatible with a diagnosis of tuberculosis even though *M. bovis* was isolated.
7. Each of the 24 herds had specimens some of which were reported as compatible and some not suggestive of tuberculosis.

It must be remembered that the tissues examined by the pathologist and the bacteriologist are not identical. In some animals specimens may

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**LABORATORY FINDINGS WHERE 10 OR MORE SPECIMENS PER HERD WERE EXAMINED FOR TUBERCULOSIS**

<table>
<thead>
<tr>
<th>BACTERIOLOGY</th>
<th>NUMBER HERDS</th>
<th>HISTOPATHOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>COMPATIBLE AND NOT SUGGESTIVE</td>
</tr>
<tr>
<td><em>M. bovis</em> and NO ISOLATIONS</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td><em>M. bovis</em>, <em>M. avium</em>, and...</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>M. bovis</em>, Runyon III and...</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>M. bovis</em>, Runyon IV and...</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td><em>M. bovis</em>, <em>M. avium</em>, Runyon II, and...</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>M. bovis</em>, Runyon II, Runyon IV, and...</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><em>M. bovis</em>, Runyon III, Runyon IV, and...</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>M. bovis</em>, <em>M. avium</em>, Runyon III, Runyon IV, and NO ISOLATIONS</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>24</td>
<td>19</td>
</tr>
</tbody>
</table>

U.S. DEPARTMENT OF AGRICULTURE FISCAL YEAR 1964 AGRICULTURAL RESEARCH SERVICE

Figure 8
not have been selected from the same gland or organ for each examination. The findings are in agreement with our recommendations that multiple specimens should be submitted to the laboratory where several animals are showing lesions, until such time as a definitive diagnosis has been made. The probability of the laboratory obtaining an $M. bovis$ isolate or a compatible histopathologic examination increases with the number of lesions submitted from each herd.

Bacteriological studies on 2,361 specimens and histopathological studies on 2,439 specimens were reported by the National Animal Disease Laboratory during Fiscal Year 1965.

**Swine**

The percentage of swine found with tuberculous lesions continue to decline. Of the 68.5 million swine slaughtered under Federal Meat Inspection last year, 1.2 million or 1.9 percent were reported with lesions of tuberculosis. $M. avium$ is considered to be the main cause of tuberculosis in swine.

During the year there was a sudden onset and rapid spread of tuberculosis in a herd of swine in a Northeastern State. $M. bovis$ was suspected as the causative agent for this outbreak. This was confirmed by laboratory examination. Epidemiological studies of this outbreak point to the feeding of improperly cooked bovine offal from tuberculin reactors as the probable source of infection. The entire herd of swine was slaughtered.

Bovine type tuberculosis was confirmed in a herd of swine in a Western State. Local laws are inadequate to require that this herd be handled in a manner that will assure control and prompt elimination of the infection.

Extensive tuberculous lesions were reported in a hog slaughtered under the local inspection system in a Southeastern State. Tuberculin tests applied to the herd of cattle that associated with the tuberculous hog revealed four reactors with advanced tuberculosis. Laboratory findings point to $M. bovis$ as the causative agent in this herd of cattle.

These three outbreaks emphasize the need for giving more attention to identification of slaughter swine and the traceback of those found tuberculous.

Data is entirely inadequate to speculate as to the prevalence of $M. bovis$ in swine.

While we are thinking and working toward zero infection and tuberculosis free areas much greater attention must be directed toward finding and eliminating all tuberculosis regardless of the specie involved.

There are presently twelve federal epidemiologists who have received or are in the process of receiving advanced training in tuberculosis at various Universities. Each of these men, as a part of his training, is carrying out intensive field investigations on a particular aspects of the technical problems which face the tuberculosis eradication program.

A preliminary study was recently finished on the use of the comparative cervical test. Results were sufficiently promising to justify a much broader study by six epidemiologists. This study will be conducted in
addition to individual projects. Individual projects in progress or in the preparatory stage are as follows:

1. The significance of blood titers in presumably negative animals on complement fixation and gel double diffusion tests is being evaluated. Preliminary results suggest that the tuberculin test produces an interference with results of serological tests.

2. Preliminary work on a milk serum test as a diagnostic or screening device looks promising. However, it will be at least two years before the procedure can be completely evaluated.

3. Organisms presumed to be responsible for non-specific sensitivity and the production of autogenous tuberculins for use on a comparative basis for differential diagnosis are being studied. There are two separate projects being conducted on this problem. Both studies have proceeded to the point where tuberculins have been produced and are ready to be evaluated.

4. The relationship of avian and swine tuberculosis to tuberculin sensitivity in cattle is under study. No significant results have been accumulated in this study to date.

5. The role of skin lesions in tuberculin sensitivity is under consideration. A protocol will be prepared on this problem and the study initiated this year.

6. A project on the effect of metabolic disorders on the projection of the hypersensitive state in cattle has been set up. Essentially this is a study of trace mineral imbalance and tuberculin sensitivity.

7. The occurrence of atypical mycobacteria in milk from cows demonstrating prolonged non-specific sensitivity is being investigated.

8. A study on the value of Johne's vaccine is in the planning stage.

9. The evaluation of the whole blood rapid ehmaglutination test for tuberculosis in chickens as a mass screening test is also in the initial planning stage.

Progress toward the eradication of tuberculosis is being made. Any relaxing of efforts must be avoided. Constant vigilance in seeking out new centers of infection is essential to the program.
THE CONTROL AND ERADICATION OF JOHNE'S DISEASE

Pierre A. Chaloux, V.M.D.
Hyattsville, Maryland

Johne's disease is one of the most controversial problems with which the regulatory veterinarian is faced today. Although State-Federal Co-operative eradication programs exist, little work is actually done. This reflects a general lack of confidence in the programs as they are presently conducted. The wide variety of action taken, sometimes within the same state, when the disease is encountered also demonstrates a basic lack of confidence in the programs.

The etiologic agent was first described as an acid-fast bacillus by Johne and Frothingham in 1895. Then in 1906, Doctor Bang of Denmark described successful experimental transmission of the disease and suggested it be called paratuberculosis. Perhaps it is unfortunate that Bang chose this name since the disease is not tuberculoid in nature. It is difficult to evaluate how much this name has had to do with the action taken against the disease down through the years. Attempts to deal with Johne's disease along the same lines as have been successful with tuberculosis have not been satisfactory. Therefore, a review of our attitudes and programs is overdue.

Johne's disease, though primarily a disease of cattle, has also been described in this country in sheep, reindeer and irradiated monkeys. Though the organism was demonstrated microscopically it was not isolated from the latter. In other countries the disease has been described in sheep, goats, and reported in reindeer, camels and captive wild animals in addition to cattle.

It is generally accepted that infection takes place through ingestion, however, congenital infections may occur in utero. Russian workers have suggested the possibility that infected bulls shed the organism in the semen. They were able to demonstrate acid-fast bacilli similar to M. paratuberculosis in the semen of infected bulls.

One of the aspects of Johne's disease that makes it difficult to study is its long latent period and the fact that infection is not always followed by clinical disease. More often it results in a carrier state. It is the sub-clinical spreader that makes eradication difficult. Much work has been done in this country and abroad to develop a reliable diagnostic procedure for use in the eradication effort. The results of this work were probably best reviewed and summarized by Pearson in 1962 as follows: "New antigens, johnins and methods of testing are being studied in a number of countries but with the high incidence of infection in so many of them, the diagnosis of carriers may at present be considered to be of secondary importance. In Britain, for example, an overall known incidence of 15 percent, would, in the absence of a satisfactory diagnostic test, justify the use of vaccines or perhaps some research into therapeutic
measures of control, but in countries where the disease incidence is low an efficient means of diagnosing infected stock is urgently required."

Sigurdsson\textsuperscript{20} concluded after an extensive review of the eradication program in Iceland that the chances of eradicating the infection from a given area by test and slaughter methods are remote and the relative efficiency of such procedures diminishes as the morbidity rate decreases.

In Denmark Ringdal\textsuperscript{21} found on an individual herd basis that it was possible to reduce the number of clinical cases but not to achieve eradication through use of isolation with test and slaughter of reactors.

In view of the nature of this disease it is time we ask ourselves whether Johne's Disease is the concern of regulatory veterinary medicine or more properly a problem for the private large animal practitioner.

What must we know about a disease in order to eradicate it?

I suggest for your consideration the following:

1. The disease must have a base for popular support. That is, it must have public health or economic significance. Johne's Disease has no public health significance and the economic aspects are submerged by the chronic nature of the disease. Death losses of 0.5\textsuperscript{22} to 15 percent\textsuperscript{23} do not seem significant when spread out over the year except to owners of large herds who keep very good records.

2. There must be a diagnostic procedure available that will detect most cases of the disease including asymptomatic spreaders.

The diagnostic procedures presently available are sometimes successful in early infections. However, in badly infected herds, a test and slaughter type program is seldom successful. Even if the entire herd is eliminated, there is no way of assuring that clean stock will be purchased.

3. Preventive measures must be available.

A vaccine may be used which will reduce the incidence of infection to a low enough level so that a test and slaughter program can be conducted on an economically sound basis for the average cattle owner.

Vaccines have been tried\textsuperscript{5,24-29} in Europe and to a lesser extent studied in this country.\textsuperscript{30,31} Extensive trials in the United Kingdom have shown that clinical disease can be reduced by 88 percent.

Separation of calves from their dams at birth is also effective especially if calves from clinical cases are not kept.

4. Treatment must be effective.

Treatment of Johne's disease has been uniformly discouraging. The only exception to this is the claims made by the French\textsuperscript{32,33,34} that correction of phosphorus deficiency in the feed will result in disappearance of clinical disease.

In view of all that we know about Johne's disease, we should seriously consider some drastic changes in our present programs. The present situation has the effect of penalizing the honest cattleman by placing his
herd under indefinite quarantine without offering too much hope of actually eradicating the disease before eradicating the herd. There is lack of uniformity in diagnosis, quarantine procedures carried out, indemnity provisions and disinfection procedures.

If we believe that the negative herd owner must be protected from infection then we should take steps to see to it that he is. At the same time we must offer the infected herd owner a reasonable program. Past experience has clearly demonstrated that this can best be accomplished by the development and adoption of a Uniform Methods and Rules.

A diagnosis of Johne’s disease should be made only when the causative agent has been previously demonstrated in the herd. In such herds, clinical disease, a positive intradermal johnin or complement-fixation test should be considered diagnostic. When Johne’s disease is first diagnosed in a herd a decision should be made on the basis of degree of infection and history whether an attempt to eradicate by test and slaughter is practical. Serious consideration should be given to the use of vaccine in badly infected herds. Though vaccination will interfere with the tuberculin test, we need to consider whether it is worse to contend with known vaccination or unknown infection in making a differential diagnosis. Naturally vaccination if used should be strictly controlled as it is in Britain. To make it practical, however, we will have to allow free movement of tuberculin negative vaccinates.

The effect of feeding mineral supplement should also be given a fair trial as a means of reducing clinical disease. Owners of known infected herds should be encouraged through indemnities to eliminate confirmed clinical cases and their last progeny immediately from the herd to slaughter. Indemnity should be restricted to owners of herds with a laboratory confirmed diagnosis of Johne’s disease. Indemnity should also depend upon complete cooperation including thorough cleaning and disinfection under veterinary supervision.

The alternative to developing a sound uniform program should be the decision to remove the disease from the concern of regulatory veterinary medicine. Such a decision must necessarily take into consideration the effect of interstate movement of animals known to be infected or exposed.

Something constructive can be done about Johne’s disease and we are long overdue in doing it. We can stop trying to deal with paratuberculosis as though it were tuberculosis. We can make a survey of slaughterhouse cattle to determine the true incidence of the disease. We can develop uniform procedures for handling Johne’s disease that are commensurate with the facts. We can conduct extensive field trials into diagnostic procedures, vaccination and treatment. All that is needed is a positive open minded approach. The United States Livestock Sanitary Association has a long tradition for generating and developing this kind of action. The veterinary profession and the livestock industry are looking for our guidance.
REFERENCES

CONTROL AND ERADICATION OF JOHNE'S DISEASE

REPORT OF THE COMMITTEE ON TUBERCULOSIS
AND PARATUBERCULOSIS


1. The committee reviewed the Uniform Methods and Rules for Tuberculosis Eradication and agreed that no changes were needed at this time.

2. At the mid-year meeting of the Committee in Chicago the question of feeding poultry litter to cattle was discussed. Dr. W. L. Mallman was asked to study the matter and furnish information to the committee at the annual meeting and to make recommendations on what must be done to render poultry litter safe to feed to cattle from a tuberculosis standpoint.

Doctor Mallman reported that it is the general feeling that this is hazardous and should be discouraged. If it is to be so used, it should be heated to a temperature sufficient to destroy pathogenic organisms. The committee endorses this recommendation.

3. At the mid-year meeting, Drs. A. F. Ranney, C. E. Kord, and E. A. Murphy were requested to contact the Packers and Stockyards Division of Consumers and Marketing Service relative to a requirement that stockyards apply back-tags as part of their marketing service.

This sub-committee reported that the Packers and Stockyards Division was not in a position to require that stockyards apply back-tags as a part of their marketing service unless such identification was required by the state or federal government. This committee recommends to the Committee on Stockyards, Markets and Transportation that a feasibility study be made relative to the identification of animals for the purpose of assisting in disease control and eradication. Special emphasis should be given to the application of back-tags to all cattle over two years of age intended for immediate slaughter.

4. Last year this committee recommended that consideration be given to amending federal regulations to include indemnity payments for the complete elimination of heavily infected herds. During the past year, federal regulations were amended to permit payment for
non-reactor animals under certain conditions, not to exceed $100.00 for grade cattle or $200.00 for purebred cattle. The committee now urges all states that do not presently have authority to pay indemnity for non-reactors in heavily infected or Red Flag herds to gain this necessary authority.

5. The need for maintaining routine testing in areas with a relatively high degree of infection is recognized. Such testing should be supported until adequate identification and traceback procedures have been established.

6. It is recommended that state and federal livestock sanitary officials at all levels maintain close liaison with public health officials and tuberculosis associations to stimulate greater interest in tuberculosis eradication for the protection of man and animals.

7. The desirability of establishing Uniform Methods and Rules for a tuberculosis eradication program for swine and poultry was considered. It was decided to defer action on these programs pending further consideration by the Tuberculosis Committee and until such time as the matter can be reviewed by the Committee on Transmissible Diseases of Swine and the Committee on Transmissible Diseases of Poultry.

8. The committee recognizes the fact that there is need for a more economical method of reaccrediting areas and determining the disease incidence. The reaccreditation of areas on a state-wide basis was reviewed. The committee feels that this can be accomplished within the scope of the present Uniform Methods and Rules.

9. The committee urges the state and federal livestock sanitary officials to consider the need for developing the identification and trace-back program to a high degree of efficiency. The probability of finding tuberculosis and the identification necessary to find a specified percentage of infected herds is contained in Tables I-IV attached.

10. The committee reviewed the problems associated with the control and eradication of Johne's disease. Plans are being developed to hold a seminar on Johne's disease at the mid-year meeting of the Tuberculosis Committee in Chicago.

11. The Tuberculosis Committee again wishes to commend state and federal meat inspection services for their contributions to the eradication of tuberculosis and urges state and federal disease control officials to continue to include meat inspection personnel as a part of the tuberculosis eradication team.

12. The Tuberculosis Committee recommends that extensive research on tuberculosis be continued and urges the Committee on Programs and Policy to stress the need for funds to continue this necessary research.
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<th>Number in herd</th>
<th>Herd Infection rate of</th>
<th>Number animals to slaughter during a 3 year period</th>
<th>Probability of finding tuberculosis</th>
<th>Slaughter Animal Identification rate of 40%</th>
<th>60%</th>
<th>80%</th>
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<tr>
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<td>10%</td>
<td>5</td>
<td>1/2</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
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<td></td>
<td>5%</td>
<td></td>
<td>1/2</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
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<td>21/2</td>
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<td>89</td>
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<td></td>
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<td>20</td>
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<td>84</td>
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<td></td>
<td>1</td>
<td>40</td>
<td>60</td>
<td>80</td>
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TABLE II

Probability of Finding *M. bovis* Infected Herds During a Three Year Period
Under the Animal Identification Program

Based on 100 percent herd turn-over during the three year period

<table>
<thead>
<tr>
<th>Number in herd</th>
<th>Herd Infection rate of</th>
<th>Number animals to slaughter during a 3 year period</th>
<th>Number lesion animals to slaughter during a 3 year period</th>
<th>Slaughter Animal Identification rate of</th>
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<tr>
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<td></td>
<td>40%</td>
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<td>1</td>
<td>40%</td>
</tr>
<tr>
<td>20</td>
<td>10%</td>
<td>20</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td></td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>50</td>
<td>10%</td>
<td>50</td>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td></td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td></td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>100</td>
<td>10%</td>
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<td>99.6</td>
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<td></td>
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<td>88</td>
</tr>
<tr>
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<td>2%</td>
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<td>2</td>
<td>64</td>
</tr>
<tr>
<td>Number in herd</td>
<td>Based on Herd Infection rate of</td>
<td>Number animals to slaughter during a 3 year period</td>
<td>Number lesion animals to slaughter during a 3 year period</td>
<td>Rate of finding infected herds 50% 75% 90% 95% Animal identification rate that is necessary</td>
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<td>---------------</td>
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<td>-----------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>10</td>
<td>10%</td>
<td>5</td>
<td>1/2</td>
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<td>20</td>
<td>10%</td>
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<td>1</td>
<td>100%  80  90  100</td>
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<tr>
<td></td>
<td>5%</td>
<td></td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>10%</td>
<td>25</td>
<td>2 1/2</td>
<td>44  60  68  96</td>
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<td></td>
<td>1</td>
<td>76  92  96</td>
</tr>
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<td>1/2</td>
<td>100%  -  -  -</td>
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<td>5</td>
<td>24  36  44</td>
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<td></td>
<td>4%</td>
<td></td>
<td>2</td>
<td>50  68  78</td>
</tr>
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<td></td>
<td>2%</td>
<td></td>
<td>1</td>
<td>76  90  96</td>
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TABLE IV

Amount of Identification Necessary to Find a Specified Percent of *M. bovis* Infected Herds During a Three Year Period Under the Animal Identification Program

Based on 100 percent herd turn-over during the three year period

<table>
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<th>Number in herd</th>
<th>Based on Herd Infection rate of</th>
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<th>Number lesion animals to slaughter during a 3 year period</th>
<th>Rate of finding infected herds 75%</th>
<th>90%</th>
<th>95%</th>
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<td>70</td>
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<tr>
<td></td>
<td>5%</td>
<td></td>
<td>1</td>
<td>75</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>50</td>
<td>10%</td>
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<td>5</td>
<td>24</td>
<td>36</td>
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<tr>
<td></td>
<td>4%</td>
<td></td>
<td>2</td>
<td>50</td>
<td>68</td>
<td>78</td>
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<td></td>
<td>2%</td>
<td></td>
<td>1</td>
<td>76</td>
<td>90</td>
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<tr>
<td>100</td>
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<td>29</td>
<td>43</td>
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<td></td>
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CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS

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<td>and E. P. Pope</td>
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DIAGNOSTIC PROCEDURES FOR CLOSTRIDIUM INFECTIONS

Earl M. Baldwin, Jr., BS, DVM, MS
Elkhorn, Nebraska

The policy of the diagnostic laboratories with which I have been associated has been to render a diagnosis, even though tentative or presumptive, in the shortest time possible. Because of the devastating effect of some clostridium infections, such a procedure is particularly important. Attempts to be too definitive can result in extensive delays and unnecessary livestock losses. The information to be presented in this paper has been formulated to provide the most expeditious methods of arriving at a diagnosis. Admittedly, some of the methods can be refined and extended, but the resulting delay in diagnosis does not warrant such procedures.

Relatively few diagnostic laboratories make a concerted effort to isolate and identify the pathogenic clostridia. Some of the most frequently encountered problems are listed below.

1. Tissue specimens are so grossly contaminated with other bacteria that examination is complicated or impossible.
2. Sporadic receipt of specimens necessitates the trouble of setting up special medias and equipment.
3. Improper and inadequate equipment is being used.
4. Odors are aesthetically objectionable.

Many practical details regarding procedures, media and equipment will be found in the 1959 report of the Committee on Anaerobic Bacteria. Another worthwhile reference is the text of Smith. It is not the intent of this paper to duplicate the text of the 1959 report, but to describe diagnostic means and methods which the author has found to be effective and practical. No single procedure can be described that will function routinely. The diagnostician must learn to adapt and modify his methods as circumstances indicate.

Pathogenic Clostridia fall into two general categories; those that actively invade the hosts tissues and those that do not invade the tissues but owe their pathogenicity to potent exotoxins. Each group requires separate isolation and identification methods and will be treated separately.

THE INVASIVE CLOSTRIDIA

Belonging to this group are *Cl. Chauvei, Cl. hemolyticum, Cl. novyi, Cl. septicum, and Cl. sordellii (Cl. bifermentans).* Culturing techniques and animal inoculation tests are employed in their identification. The

*It is the opinion of the Author that too little is known of Cl. sordellii and Cl. bifermentans to attempt to differentiate one from the other.
following list of precautions must be followed to insure satisfactory re-
sults:

1. Do not use a water washed gas to achieve anaerobic conditions. It may contain oxygen. Use a pure dry gas if available, otherwise pass the gas through a wash bottle of alkaline pyrogallol to remove the oxygen. Nitrogen is the gas of choice.
2. Gram stains should be prepared using 95 percent alcohol as a destaining agent. The Clostridia are easily destained by the destaining reagents that act rapidly.
3. Do not rely upon results obtained from specimens that are partially decomposed. Several of the pathogenic Clostridia are found in the intestine and soil. They can become post mortem contaminants. This is particularly true of liver specimens.
4. Check the accuracy of the biochemical test media regularly by using known species of Clostridia.
5. Always exercise aseptic measures in handling specimens and cultures. Laboratory facilities once contaminated are very difficult to clean up.
6. Do not use mature guinea pigs for inoculation tests. Latent infections may be activated in them and give erroneous findings. Use clean stock weighing approximately 300 to 400 grams.
7. Animal test cages should be sterilized. Contaminated cages can give rise to spontaneous Clostridial infections and/or immunity to them.
8. Do not endeavor to make a diagnosis from the gross lesions observed in test guinea pigs. They are not reliable. The offending organism must be isolated and identified before a diagnosis can be rendered.

Having suitable media on hand proves to be a problem. Liquid media if prepared in screw cap tubes can be stored for several months. Identification sugars must be prepared separately and added to the sugar free broth just prior to use. All sugar media and those that are not freshly prepared must be heated in a boiling water bath for ten minutes or more prior to their use. Modified Brewers media has proven to be suitable for cultivating all of the Clostridia. It has the advantage of supporting toxin production, as well.

All inoculums should be deposited in the bottom of the tubes of liquid media. It is very important that an actively growing culture be used as the inoculum. An anaerobe jar is not necessary for biochemical tests unless *Clostridium novyi* and *Clostridium hemolyticum* are being sought. They are by far the most fastidious of the Clostridia.

Properly collected specimens can be suitably shipped to the diagnostic laboratory by preserving them while in transit with either borax or refrigeration. Invariably the outside surface of a specimen is contaminated and must be avoided. Searing the outside surface with a red hot spatula and then removing that surface with sterile instruments will expose the interior of the specimen. Tissue from this area is removed
aseptically and ground in a Waring blender or in a mortar with sterile sand. A 10 percent suspension is satisfactory and the course tissue particles can be removed by filtering through gauze or by slow speed centrifugation. A Gram stain of the tissue extract will indicate the relative number and morphological forms of bacteria present and to be expected to appear in the cultures.

Three separate examinations are made of the tissue extract:

1. Cultures are made directly into modified Brewer's liquid media.
2. Streak plates are made on freshly prepared blood agar. These are incubated in an anaerobe jar. Suspicious colonies are subcultured into modified Brewer's media.
3. Guinea pigs are inoculated intramuscularly with approximately 0.5 ml. of tissue extract.

The guinea pig test is perhaps the most valuable of the examinations. Not only is the virulency of the suspected Clostridia demonstrated, but non-pathogenic contaminants, should they be present, are frequently eliminated. Most of the Clostridia will cause death or morbidity in 24 hours. Gross lesions and liver impression smears will be of diagnostic help but they are by no means pathognomonic. Cultures, both blood agar and modified Brewer's media are prepared from the livers of recently dead or sacrificed animals.

When cultures obtained from any or all of the three examinations are regarded as pure, their biochemical properties are determined. These tests will quickly make evident whether the cultures are pure.

*Clostridium novyi* and *Cl. hemolyticum* are the most difficult to identify because of their fastidiousness. This characteristic alone may indicate their presence if the case history is suspicious and in such an event, the anaerobe jar must be used for all cultures.

Distinguishing biochemical characteristics are largely determined by the reactions occurring in nitrate, indol, milk, and five sugar medias. The sugars are dextrose, lactose, maltose, sucrose and salicin. Additional biochemical determinations can be conducted in gelatin, urease, hydrogen sulfide, lecithinase, and lipase medias.

Fluorescent tagged antibody technique is being used with increasing frequency to identify some but not all of the Clostridia. Materials and information are available from Burroughs Wellcome and Co. Also available are specific antisera for use in neutralization tests, another valuable adjunctive diagnostic aid.

An infection in cattle caused by *Cl. sordellii* and *Cl. novyi* has been encountered with increasing frequency since 1959. The majority of the isolations have been made from cattle on full feed but a few have been made from range cattle. The course of the disease is extremely rapid and symptoms are seldom observed by either the owner or veterinarian. Losses range from two to ten percent, are generally sporadic, and frequently confined to a given group of cattle. The primary site of infection appears to be the lower throat and brisket area. The muscle tissue is characterized by reddening and extensive edema. When cut, the muscle
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exudes large quantities of serosanguinous fluid. The inside of the trachea is acutely inflamed. Cardiac hemorrhages and extensive pulmonary edema are frequently observed. Dead animals decompose rapidly. Isolations of either Cl. novyi or Cl. sordellii can be made from the muscle tissue. Guinea pigs will succumb to muscle tissue injections in about 24 hours.

THE NON-INVASIVE CLOSTRIDIA

Clostridium botulinum, Cl. perfringens and Cl. tetani produce lethal exotoxins and seldom invade the host's tissues.

The diagnosis of botulism entails identifying toxin(s) in the suspected foodstuff or gastrointestinal contents of the victim. Isolation of Cl. botulinum by bacteriological methods is not in itself significant because of the organisms wide distribution and frequent occurance in nature. Suspected toxin-containing materials are diluted 50 percent with saline, ground, and held overnight under refrigeration. The specimen is centrifuged and the supernatent used for inoculating guinea pigs (two ml. I.P.). For control purposes, two guinea pigs are inoculated with heated (100°C for 10 minutes) supernate. If toxin is present the test guinea pigs will develop a flaccid paralysis and die while the controls will be unaffected since the thermolabile toxin has been destroyed. Do not use mice as test animals because they are quite resistant to the botulinum toxins.

Clostridium botulinum produces several major toxins identified as Types A, B, C, D, and E. Neutralization tests with specific antisera will identify the offending toxin type.

Six types of Cl. perfringens, A, B, C, D, E, and F, are recognized. Types A, C, and D are of significance in the United States and cause enterotoxemia; Type A affects cattle and sheep, Type C affects cattle, sheep and suckling swine, and Type D affects cattle, goats and sheep. The toxins are formed in the intestine, principally the ileum, and usually under conditions of heavy feeding. Diagnosis consists of demonstrating the toxin in the intestinal contents and subsequently determining its type.

Isolating a toxogenic type from the intestine does not in itself warrant a diagnosis of enterotoxemia because it is well established that the various types of Cl. perfringens do exist in a saprophytic state in the intestines of many normal animals. If the case history does indicate that enterotoxemia caused death, then a confirming diagnosis of the probable Type involved is indicated.

Specimens must be collected as soon as possible after death because the toxins will deteriorate. The gut contents of the ileum and other sites showing gross pathology should be stripped out and frozen. A portion of intestine should be placed in a separate container and refrigerated. When in transit to the laboratory the specimens must be well refrigerated.

The laboratory procedures to be followed in determining the presence of toxin and the type involved are outlined in detail by the author in the Committee Report. Typing sera is available from Burroughs Wellcome and Co.
The diagnostic laboratory is infrequently requested to isolate *Cl. tetani* because of the ease with which it is diagnosed clinically. Tissues from the suspected site of infection are frequently contaminated, making isolation a long and arduous task. Once isolated, a positive identification is accomplished by demonstrating toxin and conducting neutralization tests.

REFERENCES

VETERINARY CLOSTRIDIAL BIOLOGICAL PRODUCTS:
IMMUNIZING AGENTS

M. E. Macheak, B.A., D.V.M., M.S.

Broadly speaking, this subject is closely connected with the operations of Veterinary Biologics Division (VBD), which on August 1, 1965 was established as a separate division of the Agricultural Research Service, United States Department of Agriculture. Prior to this time the veterinary licensing and inspection responsibilities were administered by Animal Inspection and Quarantine Division. These responsibilities were detailed in the Virus - Serum Toxin Act which provides authority to prohibit the interstate shipment of veterinary biologics which are worthless, dangerous, harmful or contaminated.

Anaerobic Products Section of VBD conducts the following programs of work:

1. Examines licensee-submitted Clostridial stock cultures for purity, toxigenicity where important, and identification which includes typing of *Clostridium novyi*, Types A and B; *Cl. perfringens*, Types C and D and *Cl. botulinum*, Types A, B and C. Biochemical and serological techniques are used. This program is considered necessary since Standard Requirements for potency, safety and purity of some Clostridial biological products remain to be developed.

2. Assays commercially produced veterinary biologics to determine their potency, safety and purity. Samples of every commercially produced serial of product are sent to the VBD repository where they are available for testing. Licensees are responsible for the testing of every serial of product produced and its release to market upon completion of satisfactory tests whereas VBD check tests a percentage of these serials.

3. develops Standard Requirements which outline the materials and methods to be used in assaying and provide the basis for regulatory actions taken by the Division. Existent Standard Requirements, with the exception of the ones for tetanus toxoid and antitoxin, have been developed in the past three years. Others remain to be developed in the future. The priority for development is based upon factors such as a) the availability or lack of basic scientific information which would affect the expectation of obtaining positive results without undue prolonged effort, b) the public health significance of the product, c) the presence or absence of field reports indicating a possible lack of product potency, d) the number of serials of the product produced each year and e) the availability of laboratory and animal testing space as well as trained personnel.

Head, Anaerobic Products, Veterinary Biologics Division, National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.
4. Conducts research and development necessary to provide sufficient basic scientific information so that effective and fair Standard Requirements can be promulgated.

5. Produces and supplies to all veterinary licensees Standard Re-agents, i.e. toxins, antitoxins, toxoids and challenge materials necessary to provide correlative results when conducting replicate assays.

6. When necessary, provides licensees with Standard Assay Methods which detail materials and procedures used by VBD. They serve as a model for licensee Quality Control Departments should they choose to follow them.

Veterinary pathogenic Clostridia of importance to our livestock industry are Clostridium chauvei, Cl. septicum, Cl. novyi, Types A and B; Cl. hemolyticum, Cl. perfringens, Types C and D; Cl. tetani, Cl. sordelli and Cl. botulinum, Types A, B and C. These organisms or their products are used in the production of bacterins, toxoids and antitoxins.

Often combined products are produced containing up to three Clostridial agents or two Clostridial agents and Pasteurella species. These combinations may cause problems in potency assaying due to antigenic interference between various components. Also, because of the trend toward reduced dosages, problems may arise in providing sufficient cellular and soluble antigens due to direct physical limitations as well as interference between immunizing antigens.

Clostridium "chauvei-containing" bacterins are produced in five product combinations by 24 licensed establishments. During the past fiscal year ending June 30, 1965, more than 43-1/2 million doses containing this agent were produced.

Occasionally questions arise concerning the variance in spelling of agents as found in the current seventh edition of Bergey's Manual of Determinative Bacteriology when compared to biological products labels. This difference exists because VBD has administratively established the fifth edition of Bergey's as official terminology. This has precluded the necessity of licensees making expensive printing plate changes after each revision of Bergey's Manual.

Because of the economic importance of "chauvei-containing" products, the first experimental efforts were applied to potency studies. The value of using production stock cultures recently isolated from infected cattle was demonstrated. The importance of soluble as well as cellular antigens was re-emphasized. Tissue aggressins containing soluble antigens were an important immunizing agent until the mid-1930's. Their use was discarded in favor of whole broth cultures because they were difficult to process in the laboratory. With this change, the market trend has been toward the development of multi-component products and reduced dosages. Unfortunately for "chauvei-containing" products, emphasis on cellular antigen content was promoted with loss of valuable soluble antigens. This trend is being reversed.
VBD assays potency of "chauvei-containing" bacterins by a double-vaccination-challenge type of test using guinea pigs and a Standard Spore challenge.

The challenge method is very important in potency assaying. Spore dilutions are mixed with calcium chloride and injected intramuscularly. The calcium chloride necrotizes the muscle and sets up the proper environment for germination of the spore. However, it is absolutely necessary that the spore-calcium chloride combination be injected under the lowest oxygen tension possible to provide.

The Standard Spore challenge must contain at least 10 guinea pig lethal doses for an effective potency assay. However, most serials will easily protect vaccinates inoculated with 100 lethal doses. Ideally all the vaccinates should live; all the controls should die.

Correlation of potency assays as determined in guinea pigs has been made in two groups of 110 head and 24 head of cattle. This included the results of serum agglutination tests as well as the results of vaccination-challenge. Preliminary serum agglutination results indicate a close correlation of potency assays in the two species.

*Cl. perfringens* Type C Antitoxin is produced by three licensed establishments in a volume of more than 27,000 cc. this past fiscal year. The SR requires that each cc. of antitoxin shall contain at least 800 International beta units. Potency assay requires the use of a Standard Toxin and Antitoxin in a toxin-antitoxin neutralization method with mice as indicators of neutralization.

*Cl. perfringens* Type C Toxoid is produced by seven licensed establishments with more than 870,000 doses produced during the past fiscal year. A proposed SR to become effective January 1, 1966 will require a titer of at least 10 International beta units per cc. in the pooled serums of vaccinated rabbits. Potency assay is accomplished in a two-step in vivo method in which first rabbits are vaccinated twice and bled at appropriate intervals. Pooled serums are then titered by means of the Standard antitoxin assay.

*Cl. perfringens* Type D Antitoxin and Toxoid are produced by six licensed establishments in a volume of more than 461,000 cc. antitoxin and more than 2,325,000 doses of toxoid the past fiscal year. The antitoxin must contain at least 44 International epsilon units per cc. A proposed SR for the toxoid establishes the necessary titer in serums of vaccinated rabbits at two International epsilon units.

The immune response required to protect animals against *Cl. perfringens*, Type C and Type D infections appears to be primarily antitoxic in nature. While there are some who maintain that cellular antigens also are important, little scientific basis for their need can be found.

More extensive veterinary diagnostic efforts appear to be needed to aid livestock producers curtail baby pig losses due to enterotoxemia caused by *Cl. perfringens* Type C. While the exact incidence of this disease condition is unknown, the diagnostic efforts of Drs. Don Barnes and Marty Bergeland at the University of Minnesota Diagnostic Laboratory
indicate incidence may be relatively high. Scientifically valid evidence exists that baby pigs can be protected during the critical first week of their lives either by immunizing sows with *Cl. perfringens* Type C toxoids or by passively protecting baby pigs with Type C antitoxin. Items needed to attempt to define incidence of *Cl. perfringens* Type C are vials of monovalent *Cl. perfringens* Type B, Type C and Type D Burroughs-Wellcome diagnostic serums and some 16-20 gram white mice to be used as indicators of toxin neutralization.

Active efforts are being made to develop Standard Requirements for bacterins containing either *Cl. novyi* or *Cl. hemolyticum*. The required antigenic factor in biological products containing *Cl. novyi* appears to be primarily toxic in nature whereas with products containing *Cl. hemolyticum* it appears to be primarily cellular. Since *Cl. hemolyticum* requires such precise growth factors and environment, a major problem will be to develop a Standard Challenge which can fulfill the needs for an effective potency assay.

In conclusion, may I emphasize that potent and effective Veterinary Clostridial biological products are available which meet or exceed Standard Requirements. Every effort is being made by VBD to develop Standard Requirements for potency, safety and purity of those products where they have not as yet been defined.
A PROJECTION OF TRENDS AND REQUIREMENTS IN VETERINARY MEDICAL LABORATORIES SERVICES AND FACILITIES

Kenneth D. Weide, D.V.M., Ph.D.

I would like to preface these remarks by saying that the thoughts which I will present are my own. I can hold no one other than myself responsible for them. They are not endorsed by any administrative unit, particularly my own. I do feel quite strongly concerning many of the points I will try to make and believe that I can justify most of them.

I am sure that Diagnostic Veterinary Medicine is different in Kansas than in many other areas of the country. I do feel that most of the areas from the Rocky Mountain states to the eastern Mississippi Valley do have a considerable number of things in common.

Diagnostic Veterinary Medicine in Kansas is primarily agriculture and livestock. If we omit neoplasms and specimens submitted for rabies examination, 95 percent of our receipts are associated with food-producing animals. Overall, 80 percent of the specimens we receive are associated with some form of agriculture. My thoughts are therefore influenced by these factors and this must be taken into consideration.

This does not mean that the situation is static. Great changes are taking place in Diagnostic Veterinary Medicine. Each year we receive increased requests for assistance in diagnosis of companion animal conditions and diseases. This is in keeping with the trend toward increased pet and pleasure animal services and the change of the veterinary practice picture. Also, we are offering more services to small animal practitioners. There is increased need as well as awareness for laboratory assistance in this area.

I am personally opposed to mimicry and imitation of physicians, their services and the use of laboratory assistance as occurs in human medicine. If there is a need, this is different, but so many times there is little, if any, justification other than the fact that the "M.D.'s do it." I know a veterinarian, for example, who is very lucratively engaged in electrocardiogram studies on most of his aged patients. This may contribute to the economy but I sincerely doubt, with exceptions acknowledged, that this is contributing to good solid veterinary medicine.

The man who makes his living from livestock and related agriculture can not afford the laboratory services he needs and must have to maintain a profitable operation. The practicing veterinarian, unlike the practicing physician, can not pass on laboratory charges to the livestock producer. It is simply a matter of dollars and cents. The animal health services needed by agricultural people are essential to their livestock operations.

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Those livestock producers who do not realize this are the ones who will be part-time farmers tomorrow and the next day will be with an automotive assembly plant or selling implements.

Our veterinarians can provide day-to-day service as well as consultation and animal health programming. With few exceptions, practicing veterinarians cannot provide the laboratory back-up that is needed. The livestock producer can absorb the practitioner's costs, and make a profit doing it, but he cannot absorb the laboratory expenses if the quantity and quality of that veterinary laboratory service is as it should be. This means that we must have government supported laboratories for this purpose. I personally feel the more this can be done at the local level the better. This is, of course, at the state level.

There are, naturally, laboratory services which are not available, for a variety of reasons known to us all, at the state level. In these instances we are most appreciative of assistance by federal personnel. I am opposed to utilizing federal personnel and facilities to confirm diagnoses by state laboratories when the state laboratory is perfectly qualified and instrumented to make a diagnosis. This occurred to me within the last three weeks, and I am very unhappy about it. We feel in our laboratory that we know our limitations and weaknesses and pride ourselves in knowing when we need help and do not appreciate the federal government feeling they are the last word in the diagnosis of certain problems.

I think the commercial Veterinary Diagnostic Laboratories have in the past served a very useful and important function. They have been in a position to provide services that would otherwise not be available. I think at the present time, and in the future, the sooner these services can be replaced by competent and thoroughly staffed and equipped laboratories of a disinterested third party, who have no other purpose for existence other than diagnostic laboratory services, the better. State laboratories simply do not encounter the pressures placed on commercial laboratory personnel by their management. We do not have to diagnose a disease for which there is a vaccine.

To function properly, the well-developed Veterinary Diagnostic Laboratory must be a separate and independent unit, free from encumbrances and animosities of departments within veterinary colleges or within veterinary science departments. If the laboratory operates under the administration of the State Livestock Sanitary officials, it should be a free unit within that organization. While cooperation with other agencies and individuals is a must, a basic cadre of diagnostic personnel whose main interest and duties is diagnostic veterinary medicine is essential. Physical facilities and operating budgets should be separate and distinct.

A well developed veterinary diagnostic facility has, as its supporting pillars, four areas:

1. Pathology
2. Bacteriology
3. Virology
4. Toxicology.
Failure of development or support in any one of these areas unbalances the other three and weakens the foundation structure of the entire laboratory.

I would like to dwell a few minutes on each of these four pillars.

1. Pathology. Gross pathology services should be an integral part of all diagnostic facilities. In addition to trained and experienced personnel, a well equipped facility is essential. Such facility should be planned and developed by those who have interest and experience in this area and are the ones who will be doing the work. I emphasize work, because necropsy is work. I think it is a shame that many such facilities are planned by administrators who have little concept of the problems and have no intention of working in these areas. Loading docks, animal holding quarters, drainage, waste disposal, carcass disposal, human traffic, overhead hoists, stainless steel band saws, necropsy tables, refrigerated rooms, walk-in autoclaves, formalin and specimen container storage, dressing rooms, miscellaneous storage, etc., etc., are all very important to the man who does the work in necropsy. They are essential to proper gross diagnostic facilities.

There is no reason, where it is applicable, that diagnostic material should not be utilized for teaching and/or research. We encourage this at Kansas State. We also encourage use of diagnostic personnel for student instruction where the need exists and where that individual can contribute to his special interests and experience. This must, however, be done with moderation, as the primary duty of a diagnostic staff is to provide laboratory assistance to veterinary practitioners.

Microscopic pathologic services should be available to an unlimited extent in properly functioning diagnostic laboratories. This includes the diagnosis of neoplasms. Facilities for histochemistry should be available as well as mutually beneficial consultation privileges with other staff members and with other institutions. The diagnostic laboratory should have its own competent pathologists whose primary duty is diagnostic pathology. The laboratory should have its own slide production facilities and be as automated as possible. The latest model Duo-Autotechnicon is an excellent example of some of the later developments in equipment. A vacuum infiltrator is a valuable adjunct to processing of bone, brain and lung specimens. A cryostat is a very valuable tool, especially to those of us associated directly with a veterinary hospital. Slides should be filed and cross-indexed for future reference. Those of us who are not blessed with a photographic memory need past material available for consultation and study in the diagnosis of current problems.

Time saving devices, such as the Lab-Tek method of embedding saves technician time in blocking and filing of blocks and gives a superior product.

Funds should be available to purchase new equipment as it becomes available and is needed, and it should not be necessary to budget for such items one, two, or three years in advance.

A microscope is to a pathologist as a piano is to a pianist. Each
pathologist responsible for reading slides should have his own micro-
scope, and it should be a make of his own choosing. He should have room
in his office for the microscope on a stand that is stable and designed to
support such a delicate instrument.

Gross and microscopic pathology is not the entire answer to diagnosis
nor is it necessarily the principal area in the laboratory, but a laboratory
must have this area well developed if it is to function properly.

2. Bacteriology and Serology. Competent bacteriologic facilities are
not difficult to develop—it is the acquisition of competent technical per-
sonnel that is the secret.

All of us, I am sure, are familiar with routine procedures and facili-
ties necessary for diagnostic bacteriology. Of increased significance and
what could possibly develop into a tremendous diagnostic tool is the fluo-
rescent antibody technique for identification and species typing. I person-
ally feel that this has great significance and am most enthusiastic over
the future of this tool.

Some of the older techniques should not be forgotten. Earlier this
month, we isolated an anaerobic spore forming bacillus which killed
guinea pigs within 36 hours. It was isolated from three of seven calves
which died suddenly. It was only by the use of the string of pearls test
and a Bacillus anthracis phage that we satisfied ourselves that this
was not anthrax.

Carbon dioxide and nitrogen should be available together with safe
anaerobe jars for bacteriologic studies. Water jacketed incubators, fil-
tration apparatus, automatic autoclaves, automatic dishwashers, isolation
booths, animal colonies, and animal holding facilities, together with spe-
cialized glass ware and other apparatus are a few items of equipment
needed for a well equipped bacteriologic laboratory. Media should be
stocked in good supply and readily available.

Antibiotic sensitivity testing of isolated microorganisms provides
valuable information to the practitioner. Bacterin production for special
problems is an area which should be investigated and considered.

Special services in microbiology should as techniques for PPLO
isolation and identification, growing and identifying fungi, demonstration
of rickettsia, etc., should be considered in providing complete laboratory
facilities. The demand, area problems, and availability of referral labora-
tories will influence these decisions.

Serologic tests are valuable diagnostic tools. Each laboratory should
be alert for newly developed tests and consider using them if conditions
warrant.

There is a new serologic test, soon to be reported, for equine infec-
tious anemia. To some of us, plagued with the headaches of diagnosis of
this disease, this test will certainly be welcomed.

3. Diagnostic Virology and Serology. This field is in its infancy, but
for many diseases the techniques are sufficiently developed to make them
practical laboratory tests. Optimistically, we can expect great strides in
this area, using the combined procedures of fluorescent antibody
techniques, serologic tests, and tissue culture studies.

Glassware is one of the more expensive items in diagnostic virology, especially since it is a continuing item. Sources of cells for tissue culture, at times, present problems. Minus 70-degree freezers, ultracentrifuges, inverted microscopes, Ten Broeck grinders—are but a few examples of the expensive equipment and supplies needed.

I will not go further into this area because of the time, and I know that I would more than express my ignorance in this highly technical field. I will only say that it should be a very essential and integral part of complete diagnostic facilities and is an area which holds great potential in the alleviation of animal disease.

4. Toxicology. The area of toxicology is one without end, and one which holds more headaches than any other area. Everything which dies suddenly is poisoned. The feed company has poison in its feed; the neighbor's wife poisons Farmer Brown's pigs because of an unhappy love affair, and the county road sprayer kills cattle every time it goes out. There is hardly a day goes by but what a request is made to our laboratory to check for "poisons."

Because of possible litigation, every suspect toxicosis should be considered as one which is going to court.

An analytical chemist is needed to qualify as an expert in most areas of toxicology in diagnostic veterinary medicine. It would be ideal to have someone who is both a veterinarian and a chemist. Unfortunately, these people are few and far between.

Many chemical procedures are long, detailed, and time consuming. They require instrumentation which runs into thousands of dollars. For example, a gas chromatograph is needed for most of the newer pesticides and this is in the neighborhood of $10,000, plus accessory equipment.

I am not a chemist, and cannot begin to list the equipment and instrumentation needed now or visualized for the future. I can only say that toxicology must be a part of any adequate veterinary diagnostic facility, and it must be supervised by someone who can qualify as an expert witness. Toxicology is a big field, and there is just no doubt that it will get bigger and bigger.

There are a number of items which I would like to lump together as miscellaneous. I feel they are important functions of adequate diagnostic facilities. I refer to them as braces for the supporting pillars.

I have already mentioned teaching and research. Who is more qualified to instruct students on hog cholera than one who is constantly exposed to the disease and is responsible for its diagnosis? I believe the question is self-explanatory.

I very sincerely believe that involvement in research makes a better diagnostician. Mainly because it teaches that individual to doubt, to be suspicious, and to be less positive in his approach to a particular problem. So I would promote research, again with moderation, for all diagnostic personnel.

Field investigations should be an integral part of diagnostic services.
They may be combined with state and/or federal personnel or with clinical staff of a veterinary college, or may be made direct with the veterinary practitioner. They should not be made except at the request of the local veterinarian. We should not find ourselves in the position of supplying service direct to the public.

Telephone consultation should be available to veterinary practitioners and regulatory officials 24 hours a day. This necessitates time being made available for diagnostic personnel to study and keep up with current literature in their interest area. This cannot be done with so few numbers of laboratory personnel that all that can be done is to get the work completed and report it out in time for three other problems to present themselves.

I feel very strongly that each laboratory, in as much as it can, should have training facilities to produce more and better veterinary laboratory diagnosticians. The laboratory should not, however, be run by a temporary staff or those who are using the diagnostic laboratory position as a means to another end, such as getting through graduate school.

Adequately trained and equipped secretarial staffs are a must. Automatic envelope addressing, copying machines, NCR forms, IBM data processing and use of computers for record keeping are available to us if we would but use them. It does no good to have excellence in diagnostic procedures if the report doesn't get out or if the information is not available for analysis.

I would like to close with the thought that a veterinary diagnostic laboratory, in order to be complete and adequate, need not have the resources and personnel and instrumentation to do everything. But it should have the basics—pathology, bacteriology, virology, and toxicology. An outside source, utilizing other personnel, other laboratories, or any other agency to conduct those tests and examinations and fill in those areas that are less needed and receive fewer requests is quite satisfactory.

I feel there is a great and rewarding future in diagnostic veterinary medicine. I am happy I am in it, and I hope you are, too.
REFERENCE LABORATORIES

Don E. Lundholm, D.V.M.

The once relatively static animal and poultry population has been greatly altered by changed husbandry practices and modern transportation facilities. We now face a highly mobile and transient animal population capable of confronting us with heretofore unencountered disease problems. Many problems exceed the facilities and capabilities of our laboratories as they stand today. Tomorrow, this may well be compounded. Conceivably, one could expand existing facilities and personnel, in an effort to cope with these changes. However, it is doubtful that any one laboratory could succeed to the point where they could handle everything.

Universities and veterinary schools are restricted in their diagnostic efforts by way of teaching budgets, research programs and academic responsibilities.

Ideally, all laboratories could participate in a cooperative program to provide a maximum of diagnostic service with a minimum of physical outlay. Our Western States group has initiated such a program, which, at this time, is advancing quite satisfactorily. We have participating laboratories in California, Idaho, Kansas, Nevada and Wyoming. I understand there is a similar program being initiated or functioning in the southeastern states. It is our belief that a disease condition or procedure commonly encountered and routinely handled by one state, could present problems to another, due to rare occurrence. So it makes sense to utilize each other's facilities to the utmost for the greatest benefit to all. This program, however, is not the answer, because not everyone will participate, for administrative, financial, or other reasons.

This should illustrate there is need for diagnostic reference laboratories where one can send samples or receive assistance in specific areas. The Federal Government is certainly to be congratulated for its animal disease laboratory at Ames. Certainly, we have all benefitted from the staff and facilities at N.A.D.L. The group at Ames cannot handle everything, therefore, regional laboratories should be established to complete the reference need. These should be complete diagnostic laboratories with qualified people in charge of each department—bacteriology, virology, pathology, toxicology, biochemistry, and so on. The new laboratories should be regional in nature, rather than central, so the personnel may become familiar with the problems existing in the area they service. For example, how could a laboratory in Georgia be expected to understand the problems and conditions which exist in our Great Basin area? We have poisonous plant problems, mineral deficiencies and excesses, and disease conditions peculiar to
various regions that are routine to the area, but would be difficult problems to people from a different part of the country.

The role of the reference laboratory would be to supply assistance to the State laboratories in the region it serves. When a State diagnostic laboratory has come to an impasse on a disease problem, and has neither the facilities nor money to carry it further, it may turn to the Reference Laboratory for help. It can provide services that the individual State laboratory cannot afford. For instance, many State laboratories have no tissue culture facilities, and as a consequence, are unable to carry on virus work. The workload in this field is usually light and sporadic so the costs of cultures, trained personnel, etc., cannot be justified. However, the combined samples from seven or eight states, would certainly justify this program at a regional reference laboratory, and probably at about one-tenth of the cost, and ten times the accuracy, than if everyone was trying to do it on his own.

The regional laboratory should have a trained investigational team to help on field outbreaks. Our Nevada State Laboratory functions in a manner that has been most satisfactory. We work very closely with the practitioners and the ranchers, and when they call, concerning a particular disease problem, our diagnosticians will go to the ranch. In this manner, we obtain the best possible information. We see the conditions, get good histories. In short, we get the whole picture - the live animals, the sick ones, post the dead ones, pick our own samples, and return to the laboratory and process them. In this manner, field diagnoses agree with the laboratory findings, and embarrassing disagreements due to lack of proper communication and poor samples are eliminated.

The Reference Laboratory should make available certain cultures, seras, or reagents, for use by the State laboratories. Certain fluorescent conjugates would be quite valuable. The availability of these products, commercially, for veterinary use, is rather limited, and they are quite expensive. Trying to keep everything on hand just in case something pops up, is nearly impossible. As an example of this, recently, we received sick and dead chipmunks, which we suspected of Sylvatic plague. Had we been able to get Pasteurella pestis conjugate, we could have completed our identification much more rapidly and accurately.

The Reference Laboratory should have provisions for a training program. Not necessarily a formal course-type program, but, preferably, on-the-job training. I know of no better way to learn than actually doing the work and talking with the people directly concerned. Most budgets are short on out-of-state travel funds, and our technicians are usually left out. However, if we have a regional facility nearby, the money could be obtained, and we, and our technicians, could make excellent use of the teaching facilities.

If research is to be considered, it should be channeled toward improving and developing diagnostic techniques and tools that could be readily utilized by the State laboratories. There are many possibilities.
Simplified techniques in toxicological determinations, rapid stains for histopath sections, more varied conjugates for fluorescent antibody work, expanded use of fluorometric determination, just to mention a needed few. The list is long and it will continue to grow as we try to keep pace with the everchanging and complex picture of disease.

To summarize - our patterns of disease are changing, increasing the burden on already extended laboratory capabilities. We cannot expand our present facilities to fully meet the needs of the present, much less the future. We need regional reference laboratories for help with problems we can carry no farther, and in areas where we do not have the facilities. These reference laboratories would supply special materials and reagents to State laboratories and provide training for their personnel. Any research work done should be directed towards the furtherance of diagnostic methods and techniques.

Gentlemen, we needed these laboratories yesterday, we need them today, and their need will be ever more apparent tomorrow.
VETERINARY DIAGNOSTIC LABORATORY PERSONNEL QUALIFICATIONS AND BUDGET

W. L. Sippel, V.M.D., Ph.D.

Florida Department of Agriculture
Kissimmee, Florida

Personnel:

Needs for personnel will vary with the type of livestock industry, including poultry, the area, the administrative affiliation of the laboratory, whether located at a university or not, the scope of the services offered, etc. Participation in this panel gives opportunity to offer a viewpoint that has not been previously presented regarding the qualifications of personnel of diagnostic laboratories.

To provide a complete service, departments of bacteriology, virology, (possibly combined), pathology, toxicology, serology, parasitology (possibly in the pathology departments in some areas of the country), and a field consultation veterinarian are desirable. The training requirements of these people should reflect the objectives of the laboratory. If a diagnosis based on autopsy lesions alone is the only work intended, then a D.V.M. is the only qualification necessary. As one progresses from this point, additional training and/or experience is needed.

I would like to mention specifically the fallacy of using degrees as the sole criterion for ability and/or training. The many giants in the veterinary profession who have the single degree, D.V.M., are ample proof of the fact that some of our best men required no formal training beyond the veterinary degree. Regardless of the lack of additional degrees, they had extensive additional training at the bench, either under a preceptor or by self instruction. The fact is that they were trained although they did not receive a degree. Self instruction is highly gratifying and recommended to those with adequate curiosity, persistence and ambition. This constitutes training by experience and is just as effective as formal training in a degree-granting institution. The proof of accomplishment by this method of learning is based on demonstrated ability with techniques and by good publications. Mere years of service prove little in regard to ability. Diagnostic laboratory work has a close relationship to clinical medicine. For this reason, experience in large animal practice is a valuable asset to a laboratory diagnostician.

However, formal training in a recognized, degree-granting institution is currently a widely used yardstick of competence. It is a more readily understood criterion by administrators who may not have the proper appreciation for training gained in other ways.

The practicing veterinarian sends his difficult cases to the laboratory and the best trained men available should make the autopsy examinations and others called for by the history and results of the necropsy. Very few
W. L. SIPPEL

non-veterinarians are in a position to learn the diseases that possibly could be associated with a given history and set of lesions. The use of non-veterinarians in necropsy work is patently unsound and while it may be budgetarily expedient, it constitutes misrepresentation to the public. Unless laboratory veterinarians keep current with developments in the disease field by diligent review of the literature and attending meetings such as this, neither will they remain competent. Veterinarians with a Ph.D. in their specialty or with equivalent training and experience, as mentioned above, are required for the type of service practitioners and the livestock and poultry industries have a right to expect. The toxicology department should be headed by a Ph.D. biochemist. A strong background in nutrition is helpful for this man.

New, unusual or obscure diseases are continually presented to a laboratory. The percentage of these cases that will be solved, rather than passed over, is in direct proportion to the training and abilities of the veterinarians conducting the examination.

Other speakers on this subject†,* have indicated that the D.V.M.-Ph.D. must have opportunity for research. I agree enthusiastically with this thought. Certainly there is no lack of research projects available in a diagnostic laboratory bearing directly on diagnosis. Many of these are of a nature not requiring large budgets but only sufficient energy, inquisitiveness and ability on the part of the laboratory diagnostician. I hasten to add that these attributes are not confined to veterinarians with a Ph.D. Research opportunity should be available in all diagnostic laboratories. Time will be available in an adequately staffed laboratory. As a matter of fact, in some instances there will be danger of the tail wagging the dog as research can become the primary interest of an individual whose stated mission is diagnosis. A happy and mutually profitable median can be reached under proper direction.

I very sorrowfully admit that the attitude extant among most veterinary administrators regarding diagnostic laboratory personnel differs in varying degree from those stated above. The primary reason is budgetary.

Budget:

This brings up the all important subject of funds needed to operate a diagnostic laboratory. For the benefit of those currently establishing a laboratory, I would urge you to seek advice from those operating the type of laboratory you envision. It is much easier to get adequate construction funds and operating budget in the beginning than to get increases later.

Certainly the salaries available must be commensurate with the training and experience of the personnel employed. A good diagnostic laboratory cannot be operated for any less than a good research laboratory as the same type of personnel, equipment and supplies are needed in both types of institution—with certain expensive differences on both sides. Regarding salaries, it is obvious that those in a diagnostic laboratory should be equivalent to those in the state veterinary research institution—the laboratory director paralleling the department head and the senior diagnosticians at the associate professor or professor level. The validity
of this premise is substantiated by the loss of diagnostic laboratory personnel to universities or the Federal Government at these levels.

Regarding additional staff, each department will require one or more technicians to perform routine tests and make the preparations required. The more formal training these people have the greater will be their usefulness. Registered medical technicians are adequate. Busy departments will need professional personnel at the assistant professor level. With the exception of the assistant pathologist, these individuals need not be veterinarians. Persons with a B.S. or M.S. in the specialty will be able to do a creditable job under supervision. In addition, adequate secretarial help, data processors, animal caretakers, and custodians are necessary. A level of 2.5 to three supportive personnel to each professional staff member is desirable. This is comparable to the ranges cited by Hiscocks for British laboratories and industry.

Departmental operating budgets, within a laboratory, will vary between $20,000 to $50,000 per annum per department. The greater the number of departments involved, the greater the overhead.

The total department budget will fluctuate with the amount of money needed for new equipment and other permanent improvements, called capital outlay items in our budget. Such items will vary from year to year, but necessarily must be continually added as progress is made in the various fields and new equipment found necessary to keep up with progress. Unless diagnosticians are encouraged to keep up with latest advancements by purchase of equipment as indicated, they will develop a poor morale which will be reflected by the services rendered by the laboratory.

In summary, I believe that diagnostic laboratories should be staffed by the best trained men available. The salaries for these people will need to be equivalent to those paid individuals with the same training, doing similar work, as in veterinary research laboratories.

No one knows the many unsolved problems of diagnosis better than the laboratory diagnostician. They should be encouraged to investigate them and given tools to do research on these problems.

Operating budgets for these laboratories will vary with location, but in any case, will need to be adequate to enable the individual to perform diagnostic work using the best available techniques.

REFERENCES

TRENDS IN POULTRY DIAGNOSTIC LABORATORIES
Orono, Maine

For many decades the poultry industry of the United States has been considered unimportant or commonly referred to as chicken feed. However, the past two decades have revealed many changes. Recent figures show, of the total cash farm income of this country, the biggest income producers were cattle and calves worth 8.1 billion dollars, dairy products 4.8 billion, and the third largest was poultry and eggs with 3.2 billion. The lesser important phases of agriculture were hogs, feed grains and hay, cotton, vegetables, fruits, tobacco, sheep and lambs and other livestock. It is also interesting to note that government payments to agriculture amounted to 1.7 billion dollars, of which poultry received none directly.

With poultry in third place one then discovers that this 3.2 billion dollars comes 55 percent from farm chickens and eggs, 33 percent from broilers, 11 percent from turkeys, and one percent other. The significance of this is indicated by the fact that the total chicks hatched annually is about three billion, and 100 million turkey poults. To relate these figures locally, we find that the State of Maine is ninth in broiler production in the United States amounting to about 70 million annually. Our State for years has been noted for its big potato industry. However, the poultry industry leads with 80 million dollars income, potatoes 50, dairy 30, and others in decreasing value.

Not many years ago a farm flock of 1000 hens or a 5000 bird broiler flock was considered large. Today in Maine the average size of the hatching egg flock is 8,000 whereas few, if any, broiler houses under 40,000 capacity are built. With this background one can see that the problems of disease control can be equally great, and above all, any diagnosis of disease must be prompt and accurate.

Diagnostic laboratories have been outgrowths of divisions of veterinary schools. Over a period, however, many States without veterinary colleges have provided diagnostic services for the poultry industry. These have been organized under Experiment Stations in various States, in Colleges of Agriculture and the recent trends have been under Departments of Agriculture. More recently there have been organized many branch laboratories doing elementary or general work, with special techniques being done at one central laboratory. In most cases, these branch laboratories have been under the jurisdiction of State Departments of Agriculture.

When one considers the large size of flocks and the rapidity of flock turnover, the most important facet of diagnosis is Speed. A decade ago*

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broilers were marketed at 10 weeks of age. Today they are marketed at
eight to nine weeks of age and, in fact, the time is measured in days such
as 56 days of age, etc. Another important consideration is that the feed
conversion ratios are measured in hundredths of lbs. per lb. of meat
(2.05 lbs. feed to make one pound of meat). Therefore any illness that
upsets this ratio is of great economic importance.

Speed of diagnosis hinges on physical facilities available. Years ago
gross pathology would cover most situations. Today diagnosis is very
sophisticated and bacteriology and histopathology are routine necessities.
Recent trends point to the importance of fluorescent antibody techniques
for diagnostic procedures for virology. This means the procurement of
special microscopes and antigens as well as the personnel to use this
equipment.

Old techniques such as pullorum-typhoid agglutination must be main-
tained. Recent interest in salmonella surveillance indicates that extensive
serological typing of salmonellas will be necessary and this must be done
very rapidly, within one week or sooner if it is to be of value to the poultry
industry.

Years ago it was sufficient to diagnose coccidiosis; not today how-
ever; the poultryman wants to know the precise species, i.e., *Eimeria
acervulina, Eimeria mivati, Eimeria brunetti*. This means again that
highly specialized personnel are required.

Five years ago we were beginning to isolate *Mycoplasma gallisepticum*
and diagnose chronic respiratory disease. Today some states have
mass programs of $S_6$ *Mycoplasma agglutination*. New trends developing
are mass screening programs for other *Mycoplasmas* such as *M. synoviae*
in chickens and the "N" strain in turkeys. This year our University of
Maine laboratory will do 500,000 $S_6$-PPLO tests.

Other related studies include hatchery sanitation programs. With
this modern approach the air is sampled in various areas of each hatchery
and the laboratory determines the number and type of bacteria and fungi
per cubic foot of air. This may eventually be extended to the food proc-
essing industry such as chicken and turkey processing plants.

During the past ten years standard virological serology has not only
become common but a requirement in the important poultry states. For
instance, hemagglutination-inhibition tests for Newcastle disease, serum
neutralization tests for infectious bronchitis, and embryo susceptibility
for avian encephalomyelitis are routine. Now the industry is asking for
specific serology for infectious bronchitis. They want to know if the birds
are immune to the "Mass" or "L" strain of infectious bronchitis virus.
All this type of work is making a demand for tissue culture techniques
and the use of S.P.F. (Specific Pathogen Free) chick embryos.

A trend of sophistication can be noted in the State of Maine. All the
larger poultry companies employ their own veterinarian. Our diagnostic
laboratory is receiving fewer and fewer birds for necropsy. However,
there is an increasing demand for exotic serology, specific identification
of bacteria and histopathology. Therefore the diagnostic laboratory must
have specialized persons and equipment to provide this service.
It is true that the State of Maine is not significant in cattle, sheep or swine population. However, there are 94 veterinarians in the State. Of this number 24 percent are full or partly employed in the poultry industry through service, diagnosis, research or inspection work. Few practitioners of veterinary medicine realize the opportunities that the profession has in the poultry industry. In addition, there are many more technical people such as servicemen, bacteriologists, parasitologists, etc., servicing the industry.

The future may require that certain laboratories be specialized in bronchitis serology on a very rapid basis, and the same may be true for salmonella serology. This also will be true of diseases not yet discovered. Laboratories may be required to determine exact amounts of insecticides, parasiticides, fungicides, etc., in eggs and tissues.

All this seems to indicate that many laboratories either now or in the near future will have to upgrade their services, especially for speed of diagnosis and their physical plants, staff and budgets. Organizations such as the American Association of Avian Pathologists will have to encourage advanced training for diagnosticians beyond the D.V.M. from a minimum of specialized education to an M.Sc. degree or beyond, or Board specialization.

The future is bright and challenging. If we are to be of service to this great poultry industry, and to our respective States, we must be prepared to make changes, upgrade our staff and facilities, and request higher budgets to meet this challenge.
PROFESSIONAL ASPECTS IN THE PROJECTION OF TRENDS IN VETERINARY PATHOLOGICAL SERVICES LABORATORIES

H. G. Geyer, D.V.M.*

Alfred North Whitehead states, "Education is the acquisition of the utilization of knowledge... an art very difficult to impart." We all appreciate that the teaching of veterinary medicine as an art is inseparably linked to knowledge. Yet, too frequently we fail to appreciate that knowledge without the skill to use it is inert and excess baggage to the practitioner. J. C. Bailey1 states, "Skill without the continual infusion of new knowledge leaves its possessor practicing in the grip of unmodified routines, subject to boredom, and, in the end, certain of seeing the skill he uses outmoded by men able to alter, elaborate, and extend their skill with new knowledge. Therefore, in imparting this art, we have learned the hard way, that our most difficult task is the struggle to keep skill linked to knowledge in delicate balance, so that they will reinforce, and not nullify each other. They must be acquired hand in hand or in linked sequence, because that is the way they must be used or else the art of utilization of knowledge has not been gained."

The primary responsibility of pathological laboratories is to provide information. From this standpoint it matters little how they are organizationally activated or maintained. However, without purposeful and objective administration maximum efficiency will not be achieved. A laboratory organized within and administered by a veterinary college or veterinary science department will be no different than one administered by a Department of Agriculture if each is established and maintained for the same purpose—service.

Service, as used in this instance has broad connotations. It means more than acquiring histories, conducting autopsies, making multiple laboratory evaluations and the rendering of routine reports of findings. True service is represented by the art of imparting the results of these findings as meaningful information to those requesting service. It is here that skill and knowledge must be kept in balance for full appreciation how the service can be best put to use. Bailey2 alludes to the danger of imbalance as follows, "Men overdosed with knowledge discourse with prolix glibness on what is wrong in cases before them and what is needed to set matters straight again. They are then generally unable to exhibit any of the practitioners skill as how to resolve the problems they had analyzed verbally, and, what is equally discouraging, they clearly feel no responsibility for doing so—no responsibility, in short, for utilizing their knowledge."

This emphasizes the need to maintain balance between skill and knowledge to maintain an unbiased service. Thus the laboratory veterinarians should, in addition to improving their knowledge, also improve

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their skill in the application of that knowledge particularly as it relates to the practitioner. Opportunities for self-improvement should be created and encouraged by Administrators.

The complexities of today's agriculture coupled with constant scientific advances transcend the area of what was once considered the field of animal agriculture. Staffs must be learned in the complexities of the myriad of drugs and chemicals used on soil and crops and their relationship to animal health and thence to human health. The numerous feed additives and therapeutic medicaments are equally important. In addition, the ever-changing world of pathogens and their variability of effect cannot be ignored as all are intimately related in the concept of preventive medicine.

It seems equally important to avoid the connotation of bias in identifying the laboratory's function. Since the majority of material is submitted to laboratories to ascertain abnormal or pathological conditions, would it not be more appropriate to refer to them as pathological services laboratories in lieu of diagnostic laboratories? Is it rational to believe that one or more individuals of the laboratory staff, reviewing a vague to verbose history, coupled with the technical findings, can arrive at an accurate diagnosis; especially when it is not known whether the material or specimen evaluated, truly represents the syndrome existent in the affected herd or flock? Is not the real challenge one of utilizing specialized knowledge to impart information to the practitioner in a manner to supplement his skill that he may arrive at an accurate diagnosis?

If we accept this premise, then the position of the veterinarian in the laboratory will be one of growing importance. He will be constantly challenged to not only improve his knowledge and skills, but also develop the art in communicating this as usable information to the practitioner or the concerned public.

Thus far for discussion, we have presumed the existence of the laboratory. It is here that we must face reality—for areas where they do not exist there is a need to start; where they do exist, a constant need to improve. In either situation, how do we succeed? In most instances, the existing laboratories are associated with a public agency, supported by public funds. A concerned or affected public, fully appraised of the needs and benefits, is an excellent source of support.

Thus we not only have the need for these facilities but an equal need to recruit and train personnel knowledgeable in the needs and complexities of today's science. Such persons must remain knowledgeable by continual study and improve their skills, not only in scientific evaluations, but also in communications. The ability to transmit knowledge to enhance the skills of the practitioner will enhance prestige within the professional sphere. The image will be further amplified by an appreciative public as the recipient of these talents afforded through the enhanced skills of the practitioner. It should also serve as a stimulus for the recruitment of additional veterinarians to enter this challenging field. What more is needed in stimulating interest?
The challenge is incomprehensible—incomprehensible because we have limited our perspective. This is not merely a veterinary problem, this is a people problem, involving our total socio-economic environment.

There is therefore, the challenge to create a public and professional awareness of these needs—the need for public education. Since laboratories are not directly constituted for assuming the role of adult education another avenue must be sought. It is therefore, suggested that consideration be given to the utilization of veterinary extension to assist in filling this need. Lawfully constituted as that arm of education for the adult as well as the extra-college youth, it is through experience, in a position to assume a fair measure of this responsibility in cooperation with the university, the laboratory and the practitioner. It is also in a position, through direct association with the livestock industry, to enhance the understanding and appreciation of the socio-economic benefits of these professional services. By taking the broader perspective, it becomes self-evident that the key to resolving most momentous issues confronting people is the art of the utilization of knowledge.

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1. J. C. Bailey - Organizational Behavior and Administration.
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CULTURAL AND SEROLOGIC TECHNIQUES FOR THE DIFFERENTIAL DIAGNOSIS OF MYCOPLASMA IN SWINE


INTRODUCTION

The genus Mycoplasma is composed of small filterable organisms that can be cultivated in artificial media. They are unique among bacteria in that they are devoid of a cell wall, having only a minimal limiting membrane. As a result of the cell wall deficiency the organism requires performed proteins and lipoprotein complexes to multiply. These nutritional requirements have been supplied by serum but recently additional requirements have been necessary to isolate certain of these organisms. These organisms are very poor antigens because of the lack of a cell wall and therefore stimulate little antibody response especially of the protective type. The mycoplasmas as a group will usually produce a chronic type of infection which may yield to antibiotic treatment. Antibiotic treatment though, usually only suppresses clinical signs but does not eliminate the organism from the animal. At the present time there is no one medium or cultural technique that will apply to all mycoplasmas of swine so the following discussion will be divided into the various species that have been reported. These organisms are resistant to thallium and penicillin and these substances may be used to inhibit other bacteria in isolation procedures. Thallium acetate is used at a final concentration of 1:4000 and penicillin is used at a final concentration of 200 unit/ml of medium.

MYCOPLASMA HYORHINIS

Mycoplasma hyorhinis has been associated with polyserositis of swine.¹ The agent occurs frequently, as the name implies, in the nasal cavity of swine and also as a secondary invader in preexisting pneumonic lesions. In young pigs the organism produces a transient septicemia when it gains entrance into the blood stream. This organism has a strong predilection for serous surfaces and may produce severe lesions in pigs under six weeks of age. This organism also may produce arthritis as a part of the polyserositis syndrome. In all cases of M. hyorhinis induced arthritis or polyserositic there is an associated turbinate atrophy or pneumonia.¹

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CULTURAL AND SEROLOGIC TECHNIQUES

Cultural Techniques and Characteristics:

This organism can be grown by a variety of methods. Switzer\(^1\) has reported an ox heart-avian serum medium which was subsequently enriched with swine gastric mucin, and that it is the best single system for the isolation of this organism. It may also be grown in primary swine kidney cell cultures and embryonated chicken eggs.

Growth of the organism in artificial medium produces a faint turbidity with a very slight sediment (Table I). The organism is sensitive to metabolic products accumulating in the medium upon initial isolation and should be passed at 48 hour intervals. Upon further culture of the organism it becomes less sensitive to these metabolic products and transfers may be delayed. Verification of growth may be obtained by staining sediment with Giemsa technique and observing the small coccobacillary bodies which stain purple. The organism forms typical colonies on agar which tend to sink into the agar.

**MYCOPLASMA GRANULARUM**

*Mycoplasma granularum* is also found in the nose of swine rather frequently but is incapable of producing polyserositis in young pigs.\(^1\) An acute arthritis may be produced by experimentally inoculating 180 lb. pigs.\(^1\) There is no temperature elevation in either experimentally inoculated animals or field cases due to this organism. The increased fluid in the joint is yellowish-brown in color and rarely if ever serosanguinous. The affected joint surface has a velvet-like appearance due to a villous synovitis. There is no chronic phase associated with this organism and the organism will not produce a polyserositis in your pigs.

Cultural Techniques and Characteristics:

*M. granularum* is slower in appearance of growth in liquid culture than *M. hyorhinis* reaching maximum growth in five to seven days (Table I). The organism is stimulated by the addition of both yeast autolysate (0.01 percent) and swine gastric mucin (0.5 percent) to ox heart infusion-avian serum. The growth has a faint granular appearance in liquid medium. Giemsa-stained smears of fluid culture reveal spherical staining bodies as well as cocoid rod but are surrounded by an amorphous precipitate.

**MYCOPLASMA HYOPNEUMONIAE**

This organism has recently been isolated from material from swine showing typical lesions of virus pneumonia of pigs and the isolate was used to experimentally reproduce the disease.\(^2\) This disease is characterized by a chronic pneumonia which first appears at three to 10 weeks of age. A high herd morbidity but a low mortality occurs of which a dry non-productive cough is the most typical clinical feature. Pigs retain their appetite but do not grow well.
TABLE I
Various Characteristics of the Mycoplasmas that Have Been Reported from Swine

<table>
<thead>
<tr>
<th></th>
<th><em>M. hyorhinis</em></th>
<th><em>M. granularum</em></th>
<th><em>M. hyopneumoniae</em></th>
<th><em>M. hyoarthrinosa</em></th>
<th><em>M. hyogenitalium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum Growth Appears in 2-4 days</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Optimum Growth Appears in 5-7 days</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Adaptation with Increased Growth after third passage</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amorphous Precipitate Around Organisms</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Slow Appearance of Colonies</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Without Micrococcus sp. nurse Colonies (7 da)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Granular Deposit of Sediment</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smooth Deposit of Sediment</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Increase in Growth Obtained by Agitation</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reduces Tetrazolium Salts</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reduces Methylene Blue</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pathogenic for Young Pigs when inoculated intraperitoneally</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Requires one percent glucose for Maximum Growth Without Acid Production</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Produces Only Arthritis in 100 lb. Pigs or Over.</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

This Table Modified from Switzer in Diseases of Swine; Edited by H. W. Dunne, Iowa State University Press, Ames, Iowa.
Transmission experiments indicate the disease may be spread by the airborne route as well as direct contact. Pigs free of virus pneumonia when placed in a contaminated environment but free of direct contact between them and contaminated pigs remain asymptomatic until about 15 days after the doors were closed. At slaughter these pigs had typical signs and lesions whereas littermates not exposed were free of lesions. At the first indication of clinical signs (that is coughing) weight gains were reduced by half. These experiments have been conducted by the authors and results are reproducible.

It has been reported, that when migrating *Ascaris* and *Metastrongylus* sp. larvae complicate the outbreak, the lesions are more severe. Deaths may be as high as 25 percent in these cases.

**Cultural Techniques and Characteristics:**

Isolation of *M. hyopneumoniae* is accomplished by inoculation of pneumonic lung suspension into liquid medium or agar plates. Culture of this organism may be complicated by *M. hyorhinis* as a secondary invader. It has been reported that *M. hyopneumoniae* is not susceptible to Tylosin whereas *M. hyorhinis* is susceptible. Small amounts of this antibiotic may be included in the following medium for initial isolation:

- Hank's balanced saline
- 0.5% lactalbumin hydrolysate
- 0.01% Difco yeast extract
- 20% inactivated swine serum (56° for 30 minutes)
- 200 units/ml penicillin
- 25 u/Gm./ml Tylosin

The growth of the organism is apparent after two days of observing a spiral of sediment after gently shaking the tube (Table I). It has been found that three to four day intervals are the optimum for transfer of the cultures. There is a lag in growth after the third passage which disappears by the seventh passage.

Colonies appear on agar by the second day after inoculation when *Micrococcus* sp. nurse colonies are used but do not obtain maximum size until the sixth day. Growth is slow without nurse colonies and colonies do not appear until six days after inoculation.

**MYCOPLASMA HYOARTHINOSA**

*Mycoplasma hyoartrinosa* produces a chronic arthritis in swine over 100 lbs. of weight. The disease produced by this organism differs from disease produced by *M. granularum* as follows:

1. Joint fluid is almost always serosanguinous in the acute phase.
2. An initial temperature rise occurs followed by a progressive arthritis.
3. *M. hyoarthrinosa* affected joint surfaces show hyperemia of the synovial villi.
4. Perivascular cuffing of the synovial blood vessels occurs with *M. hyoarthrinosa*.

This organism differs from *M. hyorhinis* in that

1. It does not produce polyserositis in young pigs.
2. *M. hyoarthrinosa* has an absolute growth requirement for a water soluble material found in some lots of gastric mucin whereas *M. hyorhinis* does not.

The typical clinical history is as follows:

At approximately 100 lbs. body weight, the pigs develop a temperature of 105-106°F suddenly and are reluctant to move. If the pigs are forced to move, they exhibit extreme pain by a stiff gait and will lie down as soon as possible. After several days (usually one week), they appear to recover only to develop a progressive chronic lameness. This is demonstrated at first by a progressive shortening of the stride and later by an ataxia or a hobbling type gait. The pigs do not recover but apparently do have stages of remission where little or no signs are noted.

### Cultural Techniques and Characteristics:

The following medium is used to make isolations.

- **Medium 199** (Microbiological Associates)
  - 0.5 percent gastric mucin (not all gastric mucin has the growth stimulatory factor)
  - 0.5 percent lactalbumin hydrolysate
  - 20 percent horse serum (inactivated at 56°C for 30 minutes)
  - 1 percent dextrose
  - Sufficient sodium bicarbonate to adjust pH to 7.8 to 8

The medium is sterilized by filtration and dispensed into tubes. A magnetic stirring bar is placed into each tube because the organism will not continue to passage without agitation during growth (Table I). Isolations are accomplished by the inoculation of one cc of a one to five dilution of synovial fluid or a four to five ml piece of synovial tissue into 10 ml of the medium. Optimum growth occurs in five to seven days after inoculation when incubated at 39°C. This organism also produces a CPE in swine kidney cell cultures but will not grow in embryonated chicken eggs.

*MYCOPLASMA HYOGENATALIUM*

This organism has recently been isolated from a metritis-mastitis complex in sows. Previous descriptions of this complex has been reported as being caused by *E. coli* or *Streptococcus sp.*. The clinical syndrome is as follows. Within 24-36 hours after farrowing the mammary gland begins to become firm, especially at the base. This swelling progresses until 24-48 hours later, little or no milk is being secreted. At this time, a clear mucoid material, which contains white flakes, begin to flow from the vulva. The material continues to flow for four to five days but gradually gets thicker and reduces in amount. At this time the sow
apparently recovers and milk flow returns. The greatest economic loss is due to death of pigs from hypoglycemia.

**Cultural Techniques and Characteristics:**

The same medium is used as for *M. hyoarthrinosa* and characteristics are similar (Table I). Isolations are difficult from uterine excretions without filtration of the inoculum because of contamination with *E. coli* or *Streptococcus sp.* Isolation of the organism in pure culture may be made from swabs taken from the mucosal surface of the uterus.

**SEROLOGICAL PROCEDURES**

Antigens for serological procedures are prepared as follows: The organism is grown in the above described medium in 250 ml amounts. It is centrifuged at 22,620 xg in a refrigerated centrifuge for 90 minutes and it is washed with three changes of 0.85 percent saline. For the indirect hemagglutination technic (Ross) the antigen is standardized to a McFarland Nephelometer reading of 10. For the direct agglutination test 0.25 percent phenol is added to the suspended organism as a preservative in a pH 7.2 phosphate buffered 0.85 percent saline.

**Direct Agglutination:**

Antisera are diluted making two fold dilution starting with a one to two dilution in 0.85 percent saline. Antigen (0.05 ml) is placed in a well of a glass plate and 0.1 ml of the serum dilution is added to the antigen. The mixture is incubated at 40°F overnight before being read. Inactivation of the antisera prevents the reaction and the antibody can be completely absorbed out of the antisera by antigen. Complement is probably necessary in the reaction and probably accounts for loss of activity by inactivation.

**Indirect Hemagglutination:**

A given amount of the saline suspended organisms is placed in a boiling water bath for 10 minutes. The boiled organisms are cooled to room temperature and an equal amount of five percent washed sheep red blood cells are added. This mixture is incubated at 37°C for two hours in a tissue culture roller drum. At the end of the incubation period the cells are washed three times in 0.85 percent saline and resuspended to a five percent concentration. The test is accomplished by placing 0.05 ml of the altered red blood cell on a glass plate with 0.1 ml of the serum dilutions. After a 20 minute incubation period, the test is read by observing agglutination of the red blood cell.

**Antisera Production:**

Antisera production for organisms in the genus *Mycoplasma* are very difficult to prepare because of the weakly antigenic nature of the limiting membrane. The following procedures have been used to make acceptable antisera of adequate titers.
Antisera may be produced in either SPF pigs or rabbits. It is preferred that the initial inoculum be mixed with Freund's complete adjuvant. In pigs this procedure is not always necessary but it is helpful in producing adequate titers in rabbits. In rabbits an initial dose of 0.5 ml of a suspension of organisms (McFarland nephelometer 10 concentration) is sufficient. Subsequent doses of 0.1 ml are given at three to five day interval varying in number depending upon the organism until an adequate antibody titer is obtained.

In 100 lb. pigs the initial dose may be five ml given either intramuscularly or intravenously. As high as 10 ml of a suspension of a concentration of organisms comparable to a McFarland nephelometer reading of 10 may be given at weekly intervals until an acceptable titer is obtained.

SUMMARY

The cultural and serologic techniques are described for five species of mycoplasma of swine causing various diseases. There is no one cultural medium that is optimum for all species but the one described for *M. hyoarthrinosa* will grow and usually isolate all species. In most cases the clinical syndrome that the organism is associated with delineates the species. Serological procedures are described however that will allow the grouping into species on a more precise basis.

REFERENCES

TECHNICAL ASPECTS OF TISSUE CULTURE
FLUORESCENT ANTIBODY TECHNIQUE

and L. R. Lee, B.S.

The development of the tissue culture, fluorescent antibody technique (FAT) for the detection of hog cholera (HC) virus has made available to the diagnostic laboratory a reliable tool for the confirmation of hog cholera infection. A field evaluation was reported in which specimens were submitted from suspected cases of HC for examination with the FAT. Spleen or blood samples from 317 outbreaks were inoculated into susceptible pigs to confirm the results of the FAT. Of the 234 cases found positive by pig inoculation (PI) all were found positive by the FAT with the exception of eight cases. The remaining 83 cases found negative by the FAT were all negative when inoculated into susceptible pigs. In no case did a positive FAT finding fail to be confirmed with animal inoculation. The eight cases which were negative by FAT but positive by PI were attributed to a very low content of HC virus in the specimens. The correlation of the FAT with PI on the positive cases was 96.6 percent.

Since June 1, 1964, the FAT has been utilized as a routine procedure in the Virology Section, Diagnostic Services, National Animal Disease Laboratory. Demonstration sessions have been held in various locations and personnel from state diagnostic laboratories have received training at the laboratory. Problems were encountered using the technique and questions have been raised in regard to the interpretation of results in the field. It is the purpose of this paper to discuss some of these problems and describe some work performed to investigate the application and interpretation of this method.

MATERIALS AND METHODS

Fluorescent Antibody Technique

The FAT was performed according to the protocol prepared at this laboratory and available on request. The PK-15 pig kidney cell line obtained from Cutter Laboratories, Inc., was propagated in prescription bottles employing Earle's medium plus 0.5 percent lactalbumin hydrolysate, antibiotics, and 10 percent specific pathogen free (SPF) calf serum. Coverslip cultures were prepared in Leighton tubes and inoculated with blood or tissue suspension. The best results were obtained using tissue cultures that had just produced a confluent cell sheet or covered at least


From the Virology Section, Diagnostic Services, National Animal Disease Laboratory, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.
75 percent of the coverslip. To obtain good coverslip cultures, it was necessary to use a higher concentration of cells per ml. than when growing cell cultures in prescription bottles.

The use of washed coverslips improved the quality of the cell cultures. The coverslips were boiled for 20 minutes in a two percent solution of 7X detergent* in distilled water. The coverslips were rinsed six times using a forceps or stainless steel coverslip rack, boiled in distilled water, and dried with tissue paper or in an incubator if the rack was employed.

The medium was decanted from the tubes and 1.0 or 1.5 ml. of tissue suspension depending on the size of the tube was placed in each tube. The tubes were incubated at 37°C, for one hour to permit attachment of the virus and the suspension was decanted. The coverslip cultures were washed three times with Earle's medium and fresh Earle's medium with 10 percent SPE calf serum was placed in the tubes. The cultures were incubated overnight at 37°C.

Coverslips were removed and stained by the FAT. If no infected (fluorescing) cells were detected after complete scanning of the coverslips, the culture tubes were returned to the incubator and the remaining coverslips were examined the following day.

Certain points should be emphasized regarding the examination of the coverslips under ultra-violet-blue light. The microscope should be located in a dark room (a large closet is excellent) and the observer should allow his eyes to become adjusted to the dark before examining the preparation. Under ultra-violet-blue light using the darkfield condenser the negative cell sheet will be a dark brown or dark green. Positive cells which are infected with hog cholera virus will be bright green (apple green or chartreuse) and will contrast sharply against the darker, negative, surrounding cells. The nucleus does not stain and appears as a dark area in the center of the cell. The outline and protoplasmic extensions of the infected cell will be well demarcated. Occasionally cells will have a nonspecific fluorescence which will be a blue-gray color with a lower intensity. This nonspecific fluorescence may be due to dead cells or toxic cells with cytoplasmic granules. To differentiate this nonspecific fluorescence the incandescent light source is switched on the preparation. If the fluorescence observed in a cell is specific, the cell will appear no different from its surrounding cells when viewed by incandescent light. However, cells having nonspecific fluorescence will appear brighter than the neighboring cells when viewed by incandescent light.

**Fluorescent Microscopy**

The microscopic examination was performed with the Leitz Ortholux microscope** with a BG-12 blue exciting filter and the OG1 yellow barrier filter. The light source was the HBO-200W lamp and the condenser employed was the dry darkfield condenser which produces an illuminated

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**E. Leitz, 468 Park Avenue South, New York, New York 10016.
darkfield area of approximately one mm. in diameter suitable for low power, 100-300X, observation of tissue culture cells.

Care must be taken to insure proper focusing of the light source. First the focusing lens in the base under the microscope should be moved into position. The lamp image should be brought into focus on a ground glass or sheet of paper placed above the substage mirror of the microscope about one cm. to the left of the center point. The mirror image of the lamp should be located by manipulating the rod on the back of the lamp housing and brought into focus about one cm. to the right of the center point. Now with the two images in focus the screw which controls the lateral motion of the lamp base may be turned so that the two images approach and superimpose. The maximum illumination will be obtained in this manner. The focus of the lamp should be checked regularly. However, during darkfield examinations the focusing lens located in the base should be moved out of the light path so that a sharp cone or area of illumination on the microscope slide is produced.

Specimens

Swine herds suspected of infection with HC were investigated by Animal Health Division veterinarians and tissues collected from dead or moribund pigs following post-mortem examination were submitted to the laboratory. The tissues were shipped frozen with dry ice in insulated polystyrene containers by air express and usually arrived at the laboratory within 48 hours. Tissues collected from experimental pigs were stored at minus 84°C. before examination with FAT.

Pig Inoculation

Second generation, SPF pigs weighing 30-50 pounds were used in the experiments. Two experiments were performed to determine the virus content of tissues from infected pigs following infection with HC virus. In the first experiment, 24 pigs were inoculated with 0.5 ml. HC virus, Ames challenge strain, intramuscularly into the hind leg and housed collectively in the same area. Two pigs were killed each day starting with the first day postinoculation (DPI). Blood, spleen, and tonsil were collected and examined by the FAT.

In the second experiment, 38 pigs were inoculated with 2.0 ml. of a modified-live HC virus vaccine of porcine origin which was reconstituted and diluted with an equal volume of sterile distilled water. No hyperimmune serum was administered and all pigs were maintained together. Two pigs were killed each day starting with the first DPI through 12 DPI. After 12 DPI two pigs were killed on alternate days beginning with 14 DPI and continuing through 26 DPI. Blood, spleen, tonsil, mandibular lymph node, lung, kidney, brain and feces were collected and examined by the FAT. Urine was collected from 10 pigs at postmortem and examined by the FAT.
Plaque Counting and Titration of HC Virus

The virus content of blood and tissues was determined after a method previously reported for counting leptospira. The microscopic field area of the low power darkfield condenser was measured and the ratio between the area of the field and the area of the coverslip was calculated. The number of positive (fluorescent) cells or plaques of cells per field was counted in 20 randomly selected microscopic fields. The mean or average number of virus plaques per field was calculated by dividing the total count by 20. The mean number of virus plaques per field was multiplied by the conversion factor or ratio to determine the total number of virus infected cell plaques per coverslip. When the coverslip was inoculated with one ml. of tissue suspension this count represented the number of virus particles per ml. that were able to infect the tissue culture cells. This count was designated the "fluorescent antibody infectious unit count." When a small number of plaques was observed, the whole coverslip was counted.

The accuracy of the procedure was checked by placing seven tissue suspensions in coverslip culture tubes and making plaque counts after overnight incubation. The same seven suspensions were diluted serially in tenfold dilutions using fresh pipettes to transfer each aliquot. Five coverslip tissue cultures were inoculated with each dilution. The cultures were incubated at 37°C for 48 hours and the coverslips stained by fluorescent antibody conjugate. The virus titers were calculated according to the method of Kärber.

Histopathology

Frozen and paraffin sections of brain tissue were stained by the hematoxylin-eosin method and examined by the personnel of the Pathology Section, Diagnostic Services, National Animal Disease Laboratory. The detailed description of the findings will be published separately.

RESULTS AND DISCUSSION

Processing of Hog Cholera Accessions from the Field

During the routine application of the FAT to 480 accessions submitted from the field, HC virus was detected in 218 cases or 45 percent. All positive cases were detected after incubation of the inoculated coverslip cultures for 18-24 hours with the exception of 10 cases which required 42-48 hours incubation. In addition to fresh tissue submitted, brain specimens fixed in 10 percent formalin for histopathological examination were received from 249 of the total number of herds. Brain histopathology confirmed the positive FAT findings in 109 specimens or 43.8 percent. Negative FAT results were correlated with the absence of brain lesions in 89 submissions or 35.7 percent. In the remaining 51 submissions where there was disagreement, brain lesions were found in 39 cases, 15.7 percent, in which no virus was detected by the FAT. The contrary finding of a positive FAT result and no brain lesions was recorded in 12 submissions, or 4.8 percent.
The agreement in the findings was as expected in view of the fact that
brain lesions will persist for a considerable period of time after virus can
be recovered. On the other hand virus can be recovered in the early
stages of infection before characteristic brain lesions become marked in
appearance. The data indicated that both procedures should be applied to
achieve an adequate laboratory confirmation of a field diagnosis of hog
cholera.

In a study of 38 positive accessions where spleens from two pigs had
been submitted, virus was detected with the FAT in only one of the two
spleens in 15 cases. In a group of nine confirmed cases where three
spleens were submitted there were four cases in which all three spleens
were positive, three cases in which only two spleens were positive, and
two cases in which virus was detected in only one spleen out of three.
Although the coverslips from these negative spleens did not receive the
total scanning that was applied to coverslips of negative accessions, it
would seem reasonable to recommend the submission of the tissues from
at least four pigs from any herd suspected of HC infection.

In an analysis of 86 paired spleen and blood samples both spleen and
blood were found positive to the FAT in 40 cases and spleen was positive
and blood negative in the remaining 46 cases. Since nearly all of the pigs
from which these samples were collected had died of HC or were euthan-
ized in a moribund condition, it would seem that the blood was not a good
source of virus in the advanced stages of the disease. Blood samples
should be collected from febrile pigs that are still fairly active. Circulat-
ing antibody present in the blood after the pig has been sick for several
days would prevent isolation of the virus from the blood.

Accuracy of Plaque Counting Method

The plaque counts of seven tissue suspensions were determined as
described and converted to logarithms to the base 10 for comparison with
the titers determined by the tenfold serial dilution method. The results
are indicated in Table I. The mean titers calculated for the two methods

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Tissue</th>
<th>Plaque Titer (log)</th>
<th>Dilution Titer (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16556</td>
<td>Tonsil</td>
<td>3.6</td>
<td>2.5</td>
</tr>
<tr>
<td>16112</td>
<td>Spleen</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>16112</td>
<td>Tonsil</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>16362</td>
<td>Spleen</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>16672</td>
<td>Spleen</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>16672</td>
<td>Tonsil</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td>16362</td>
<td>Tonsil</td>
<td>0.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Mean Titers 2.56 2.36
were 2.56 for the plaque titration and 2.36 for the dilution titration. The variances were analyzed statistically and no significant difference was found between the two methods.

The plaque count should be designated the "fluorescent antibody infectious unit count" rather than the actual number of virus particles for several reasons:

1. The infected cells may produce additional virus during overnight incubation and infect other cells creating a slightly higher count than the number of virus particles originally present in the tissue suspension.
2. Virus may be attached to pieces of cell protoplasm and one plaque may be the result of a cluster of virus particles.
3. Some of the virus particles may be partially inactivated and though incapable of infecting the tissue culture cells could produce HC if inoculated into a pig.
4. Some virus strains may have a slower rate of multiplication in the cell and produce less plaques after overnight incubation than would be observed following prolonged incubation.

The plaque counting method was found to be as accurate as the titration procedure and was easier and less time consuming.

**Primary Pig Kidney Versus PK-15 Cell Cultures for HC FAT**

Primary pig kidney coverslip cell cultures may be used for the FAT and are prepared routinely in our laboratory as reserve cells for unexpectedly heavy diagnostic loads. However, casual comparison on the same specimens indicated that the PK-15 cell line produced brighter fluorescence and higher plaque counts. Plaque counts were recorded for three specimens which were inoculated on both PK-15 and primary pig kidney cells in which a rather sharp discrepancy was observed. The plaque counts are indicated in Table II.

The difference between the two cell cultures may be explained by the fact that the PK-15 cell line was cloned from a single cell and has uniform characteristics. The primary cell cultures may vary from one batch to another according to which type of cell finds growth conditions most favorable. Since the two cell cultures were prepared with different media and serum supplements, it was considered advisable to check for virus

### TABLE II

Plaque Counts of Three Specimens Inoculated on PK-15 and Primary Pig Kidney Cells

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Plaque Counts per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PK-15</td>
</tr>
<tr>
<td>Spleen 14563-1</td>
<td>1870</td>
</tr>
<tr>
<td>Spleen 14563-2</td>
<td>4820</td>
</tr>
<tr>
<td>Spleen 14561</td>
<td>1870</td>
</tr>
</tbody>
</table>
inhibitors in the primary pig kidney cell cultures. The specimens were inoculated on the primary cell culture after first washing the coverslips three times with Earle's medium, pH 7.4, and then incubating the cultures covered with the medium for 15 minutes to elute inhibitory material which might be attached to the cells. The FAT was performed and plaque counts were made at 24 and 48-hour intervals. The results are shown in Table III.

The plaque counts were not increased by washing the coverslip cultures. In addition, the numbers of plaques observed after 48 hours' incubation were not consistently increased over the 24-hour counts. It was concluded that the decreased susceptibility of the primary pig kidney cells was not due to soluble virus inhibitors but was inherent in the nature of the predominant cell type growing in the culture.

**Effect of Nutrient Serum Containing Bovine Virus Diarrhea Antibodies on the HC FAT**

In view of the known antigenic relationship between bovine virus diarrhea (BVD) virus and HC virus, the question was raised as to whether the presence of BVD antibodies in enrichment serum of bovine origin would interfere with the infection of the monolayers of the PK-15 tissue cells with HC virus. To determine if the results of the FAT would be affected, some serums containing BVD antibodies were obtained and passages of the PK-15 cell line were grown using these serums in the mediums. Two serums were employed, a convalescent serum with a neutralization titer of 1-512 against BVD virus and a hyperimmune serum with a titer of 1-4096. Four strains of virulent virus suspensions diluted 1-10 were inoculated into the coverslip cultures. In addition, a set of dilutions were prepared with each virus and inoculated into two coverslip cultures per dilution. After 24 hours incubation, the FAT was performed. Plaque counts were made and endpoints for the titration were calculated. The results are indicated in Table IV.

Virus strains I, II and III were compared on cells grown in the BVD convalescent serum and SPF calf serum while virus strain IV was compared on cells grown on the BVD hyperimmune serum and fetal calf serum. There was no consistent reduction of plaque or titer in the experiment which could be attributed to the BVD antibodies in the nutrient serum. The immune serums had better growth stimulating qualities for the PK-15
TABLE IV

Effect of a Nutrient Serum Containing Antibodies Against Bovine Virus Diarrhea Virus When Employed for Growth of PK-15 Cell Line Used for the FAT

<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>Plaque Counts*</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPF Calf Serum</td>
<td>BVD Antiserum</td>
</tr>
<tr>
<td>I</td>
<td>TNTC**</td>
<td>2760</td>
</tr>
<tr>
<td>II</td>
<td>364</td>
<td>65</td>
</tr>
<tr>
<td>III</td>
<td>679</td>
<td>1000</td>
</tr>
<tr>
<td>IV†</td>
<td>226</td>
<td>1000</td>
</tr>
</tbody>
</table>

*Virus diluted 1-10
**Too numerous to count
†Fetal serum vs. hyperimmune serum

TABLE V

Plaque Counts of Spleen and Tonsil Specimens of 28 Pigs

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Plaque Counts per ml.</th>
<th>Case Number</th>
<th>Plaque Counts per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Tonsil</td>
<td></td>
</tr>
<tr>
<td>13202</td>
<td>4,680</td>
<td>1,470</td>
<td>14330</td>
</tr>
<tr>
<td>13789</td>
<td>26,900</td>
<td>6,510</td>
<td>14207</td>
</tr>
<tr>
<td>13875-1</td>
<td>11,300</td>
<td>11,600</td>
<td>14132</td>
</tr>
<tr>
<td>13875-2</td>
<td>34,000</td>
<td>20,000</td>
<td>8486</td>
</tr>
<tr>
<td>14128-1</td>
<td>20</td>
<td>3</td>
<td>16112-2</td>
</tr>
<tr>
<td>14128-2</td>
<td>45</td>
<td>3</td>
<td>16112-3</td>
</tr>
<tr>
<td>14224-2</td>
<td>335</td>
<td>6</td>
<td>16112-4</td>
</tr>
<tr>
<td>14329</td>
<td>2,630</td>
<td>2,410</td>
<td>16132-1</td>
</tr>
<tr>
<td>14122</td>
<td>535</td>
<td>79</td>
<td>16132-2</td>
</tr>
<tr>
<td>14486</td>
<td>10</td>
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<td>14109-1</td>
<td>549</td>
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<tr>
<td>14109-2</td>
<td>754</td>
<td>240</td>
<td>16196-2</td>
</tr>
<tr>
<td>15751</td>
<td>32</td>
<td>12</td>
<td>16196-3</td>
</tr>
<tr>
<td>15786</td>
<td>7</td>
<td>11</td>
<td>16420</td>
</tr>
</tbody>
</table>

cells than the SPF calf serum. It was concluded that the presence of BVD antibodies in serum supplement does not interfere with the FAT.

Comparison of Spleen and Tonsil as Sources of Virus for the FAT

It was suggested that the tonsil was a better source of virus than the spleen in the HC infected pig. Although the FAT had been evaluated thoroughly using the spleen as the specimen tissue, it was decided to make a comparative study on field accessions. Diagnosticians were asked to submit a specimen of tonsil tissue with the spleen of each pig examined for lesions of HC. The tonsil tissue was processed as described and inoculated on replicate cultures with the spleen. Plaque counts were performed and the results of 28 pairs of spleen and tonsil are recorded in Table V.
### TABLE VI

<table>
<thead>
<tr>
<th>Days Post-Inoculation</th>
<th>Pig</th>
<th>Number of Plaques per ml.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>Spleen</td>
<td>Tonsil</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>0</td>
<td>4</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>713</td>
<td>17</td>
<td>567</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>--</td>
<td>892</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>--</td>
<td>400</td>
<td>545</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>--</td>
<td>4,060</td>
<td>1,400</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>--</td>
<td>2,280</td>
<td>667</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>4,290</td>
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<td>6,530</td>
</tr>
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<td></td>
<td>B</td>
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<td>7</td>
<td>A</td>
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</tr>
<tr>
<td></td>
<td>B</td>
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<td>31,200</td>
<td>5,790</td>
</tr>
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<td>8</td>
<td>A</td>
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<td>11,500</td>
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<tr>
<td></td>
<td>B</td>
<td>&gt;1,000</td>
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<td>1,430</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>7,500</td>
<td>6,270</td>
<td>29,000</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>52,500</td>
<td>22,200</td>
<td>1,430</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>32,300</td>
<td>20,100</td>
<td>2,140</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>93,400</td>
<td>13,800</td>
<td>2,880</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>132,000</td>
<td>9,920</td>
<td>1,920</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>275</td>
<td>6,690</td>
<td>436</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9,810</td>
<td>18,800</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>226</td>
<td>11</td>
</tr>
</tbody>
</table>

---Specimen not collected

The spleen counts were consistently higher. The counts were converted to logarithms to the base 10 and analysis of variance was performed. The F ratio for difference between the two tissues was 17.02 as compared with the F ratio of 7.68 at the one percent error level from the statistical table of variances. This indicated a highly significant difference between the two tissues. The logarithmic transformation was justified because the effect of virus multiplication in tissue is proportional rather than additive and the standard deviation of the plaque counts varied directly with the mean—the larger the count the greater the standard deviation.

**Virulent Virus Experiment**

The pigs inoculated with the virulent challenge HC virus all had an increase in rectal temperature on the third or fourth day. Two pigs were killed each day until nine DPI when five pigs were found dead and 10 DPI when the remaining three pigs died. The plaque counts recorded for spleen, tonsil and blood are indicated in Table VI. Virus was detected in
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the spleen of one of the pigs killed on the first day postinoculation. On two DPI virus was found in spleen and tonsil of both pigs. The plaque counts increased daily to seven DPI.

The mean daily plaque counts were calculated and presented graphically in Figure 1. The mean tonsil counts exceeded the spleen counts on the second and third day. On four DPI and throughout the rest of the experiment, the mean spleen counts were higher than the tonsil counts.

The number of pigs used was limited but some indication was given that the tonsil has a higher content of HC virus early in the course of the disease. However, spleen was a better source of virus after the fifth day following infection. The tissue culture fluorescent antibody method is an extremely valuable technique for detecting virus content of tissues with viruses like hog cholera which previously would have required extensive animal inoculation.

**Modified Virus Experiment**

The pigs inoculated with the modified live virus vaccine of porcine origin were observed to be sick on three DPI with signs of anorexia, straight tails, mucous discharge from the conjunctival sac, and reluctance to move. The rectal temperatures were increased on two DPI and reached a peak between six and eight DPI. The temperatures returned to normal over a period of several days. Several pigs had diarrhea and one pig had a bloody diarrhea. After 16 DPI the remaining pigs appeared normal.

Characteristic lesions of hog cholera were observed on post-mortem examination; however, the lesions were less severe than those observed in the pigs inoculated with virulent virus. The most marked lesions were
observed between eight and 12 DPI with hemorrhage and congestion of lymph nodes, infarcts of the spleen, and petechial hemorrhages of the kidney, bladder and epiglottis. The two pigs killed each day were those which appeared most affected.

All tissues collected were examined by the FAT and the results are indicated in Table VII. Virus was initially recovered from spleen, tonsil, and mandibular lymph node from the two pigs killed on two DPI in very low titer, one to two plaques per ml. The two pigs killed each day from three DPI through six DPI all had virus in the tissues with increasing counts. Blood and lung tissue from pig 8897, five DPI, were positive and lung tissue from pig 8874, six DPI contained virus. Only one pig had virus on seven DPI and no virus was recovered from the eight DPI pigs. The
pigs euthanized on nine DPI were positive with low counts but no recoveries were made from pigs killed on the 10th day. Two plaques per ml. were detected in the tonsil tissue from one of the 11 DPI pigs and the other pig was negative.

It was noteworthy to find a wide distribution of virus in the tissues of pig 8895 killed on 12 DPI. Virus was detected in the brain, kidney and lung as well as in the spleen, tonsil and lymph node. After 12 DPI, pigs were killed on alternate days through 26 DPI. Pig 8896, 14 DPI, had virus in the tonsil, lymph node and lung. No virus was recovered from any of the pigs killed after 14 DPI.

Virus was detected only once in the blood, two plaques per ml. on 5 DPI. Feces were collected from all pigs and urine from 10 pigs but no virus was found. HC virus was recovered from the lung tissue of four pigs and the kidney of one pig. The mandibular lymph node was found to be as good a source of virus as the spleen and tonsil. A bar graph is presented in Figure 2 indicating the relative concentration of virus isolated from spleen, tonsil and lymph node during the experiment. The counts obtained for spleen, tonsil and lymph node were studied statistically and no significant difference in the plaque counts was found among the three tissues.

The results of this experiment indicated that modified or attenuated virus is not easily recovered from pigs by the FAT. Low virus counts obtained from an ample submission of tissues from a field case may give some indication that a virus strain of reduced virulence is involved. On the other hand, high virus plaque counts found in a set of tissue specimens may give reason to suspect the presence of virulent field virus. Of
importance to the HC eradication program was the recovery of virus from pigs killed 12 and 14 days after inoculation. The suggestion may be offered that pig 8895 which had HC virus recovered from six tissues including lung, kidney and brain was in a "carrier state." Of course, if serum had been administered at the time the virus was given, there would probably have been some interference in the recovery of virus.

The pig experiments were planned to study the application of the FAT with virus strains at each extreme of the virulence spectrum. The experiment should be repeated with HC virus strains of intermediate virulence, particularly those which are not lethal and produce a lingering form of the disease without complete recovery. However, some information has been obtained that the virulence of the infecting HC virus strain may have a relation to the virus content of the tissues as detected by the fluorescent antibody technique. The importance of submitting specimens from several pigs for laboratory confirmation of a HC diagnosis was given more emphasis.

SUMMARY

The technical aspects of the fluorescent antibody technique were reviewed and some suggestions were made on how the operation of the procedure may be facilitated. A plaque counting method for the estimation of the virus content of tissues from infected pigs was described and evaluated. Pig inoculation experiments were performed with the two HC virus strains, virulent and attenuated, and the virus content of the tissues was determined throughout the course of the infection.

ACKNOWLEDGMENTS

The authors express their appreciation to Mr. Timothy Wheelock for technical assistance and to Mr. Ralph Glazier for the graphic illustrations.

REFERENCES

2. Diagnostic Services, National Animal Disease Laboratory, Coverslip Tissue Culture, Fluorescent Antibody Technique for the Detection of Hog Cholera Virus. (Mimeograph)
3. Diagnostic Services, National Animal Disease Laboratory, Serum Neutralization With Bovine Virus Diarrhea Virus (NADL PK-15 Adapted, Malmquist Strain) Employing the PK-15 Cell Line. (Mimeograph)


SERUM NEUTRALIZATION TESTS FOR BOVINE VIRAL DIARRHEA VIRUS AND INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS EMPLOYING ESTABLISHED TISSUE CULTURE CELL LINES

E. A. Carbrey, V.M.D., M.S. and L. R. Lee, B.S.

An established tissue culture cell line which is susceptible to infection with virus has many advantages over primary cell cultures.* The ease of transfer and maintenance of a cell line enables laboratories with limited resources to develop tissue culture competency.

With this in mind, Malmquist, Fernelius and Gutekunst adapted the National Animal Disease Laboratory (NADL) strain of bovine viral diarrhea virus (BVDV) to growth on the PK-15, CCL-33, cell line.** After 24 passages on the PK-15 cell line, the BVDV was producing cytopathic changes in the cells at 48 hours. At 72 hours following inoculation with this virus strain, the cell cultures were almost completely destroyed. Mixing the virus suspensions with serums taken from cattle following an infection with BVDV, prior to inoculation of cell cultures, neutralized or inhibited this cytopathic effect. The BVDV was propagated in prescription bottle cultures of the PK-15 cell line and stored in the frozen state. Tube cultures of cells were produced and serum neutralization tests performed on specimen serums submitted for diagnosis.

The MDBK, CCL-22, bovine cell line was found to develop cytopathic changes when infected with infectious bovine rhinotracheitis virus (IBRV) by Lukas et al. This cell line-virus system provided a tool for the detection of serum antibodies against IBRV.

MATERIALS AND METHODS

Tissue Culture

The PK-15 cell line was maintained according to the directions in the protocol prepared by Diagnostic Services, National Animal Disease Laboratory, Ames, Iowa, entitled "Coverslip Tissue Culture, Fluorescent Antibody Technique for the Detection of Hog Cholera Virus." However, other methods of preparing Earle's medium and related reagents have been used with equal success.

From the Virology Section, Diagnostic Services, National Animal Disease Laboratory, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

*Exhibit by the Virology Section, Diagnostic Services, Animal Health Division, National Animal Disease Laboratory, Ames, Iowa.
**Cutter Laboratories, Inc., Berkeley, California.
***Pitman Moore, Inc., Zionsville, Indiana.
The production of cell monolayers in culture tubes, 16 x 150 mm., did not require as concentrated a cell suspension as when seeding Leighton tubes. The harvested suspension of PK-15 cells from a four ounce prescription bottle was diluted to 36-45 ml. when seeding tubes or prescription bottles. An inoculum of 1.5 ml. of cell suspension was employed in seeding culture tubes. Disposable tubes (use once and discard) and tube holders were obtained commercially.*

The MDBK-20 cell line was cultured in a similar manner to the PK-15 cell line except for the use of 10 percent lamb serum as medium enrichment for propagation and five percent for maintenance. Confluent cultures were produced in four to five days.

**Virus Propagation**

Prescription bottles were seeded with the PK-15 cell line and incubated until a confluent cell sheet was obtained, usually within three to four days. The medium was decanted and the BVDV suspension was placed on the cell sheet; 1.0 ml. inoculum was adequate for a four ounce bottle. The bottles were incubated at 37°C for 15 minutes to permit absorption of the virus and then fresh medium, Earle's balanced salt solution plus 0.5 percent lactalbumin hydrolysate, antibiotics and five percent calf serum, at pH 7.6, was added without removing the inoculum.

The bottles were incubated at 37°C, and observed for the cytopathic effect (CPE) of the virus, which was apparent after 48 hours. Almost complete destruction of the cell sheet occurred by 72 hours and the tissue culture fluid was harvested at this time. The virus harvest was pooled, centrifuged to remove cellular debris and stored in suitable aliquots. The BVDV strain has been stored at -60°C for at least two months without loss of titer.

The IBRV was propagated on the MDBG cell line in the same way. However, the cytopathic effect usually developed within 48 hours.

**Virus Titration**

The suspensions of BVDV and IBRV were thawed rapidly and ten-fold serial dilutions were prepared using Earle's medium, pH 7.2-7.4, as diluent. Separate pipets were used to transfer and mix each dilution. Sufficient tube cultures were prepared so that each virus dilution was inoculated into four or five tubes. An inoculum of 0.1 ml. per tube was employed. The virus titration tubes were examined daily for CPE. The final reading was made six days postinoculation. An adequate number of uninoculated tubes of cells were maintained in the same rack with the virus titration as a control on the quality of the cell sheet and as an aid in determining the highest dilution of virus which caused destruction of the cells. Each tube was recorded as

*Bellco Glass, Inc., Vineland, New Jersey.
Arthur, Thomas Co., P. O. Box 779, Philadelphia, Pennsylvania 19105.
positive or negative for cytopathic effect. The 50 percent endpoint of the virus titer was calculated by the method of Kärber. An example is given below:

**Virus Titration**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>$10^1$</th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
<th>$10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

If $t_{50}$ = 50 percent endpoint

$t^*$ = any dilution below the 50 percent endpoint having 100 percent positive tubes

$\Delta t$ = increment to 50 percent endpoint then

$t_{50} = t^* + \Delta t$

As an approximation:

$$\Delta t = \frac{\text{Sum of the positive tubes at and above } t^*}{\text{Number of tubes per dilution}} - \frac{1}{2}$$

$$\Delta t = \frac{5 + 4}{5} - \frac{1}{2} = 1.3$$

$t^* = 10^3$

$t_{50} = t^* + t = 3 + 1.3 = 4.3$

Therefore, the 50 percent endpoint of the virus was produced when the virus was diluted $1:10^{1.3}$ and 0.1 ml. inoculated per dilution.

To determine the number of 50 percent tissue culture infective doses in the 0.1 ml. of inoculum the antilogarithm of 4.3 is calculated.

$$\log N = 4.3$$

$$N = 20,000$$

Therefore, the virus suspension contained 20,000 50 percent tissue culture infective doses, or TCID$_{50}$, per 0.1 ml.

**Serum Neutralization**

The beta method was employed in which a virus inoculum of constant titer was titrated against different serum dilutions. The serums were heat-inactivated at 56°C for 30 minutes. The virus suspension was diluted with Earle's medium, pH 7.2-7.4, so that the test inoculum contained 100 to 1000 TCID$_{50}$ per 0.1 ml. The serums were diluted according to a fourfold dilution scheme in Earle's medium, pH 7.2-7.4 and each serum dilution was mixed with an equal amount of the test inoculum of virus.
The inoculated cultures were placed in the incubator at 37° C. and observed daily. Cytopathic changes were observed in three to four days and final readings were made on the sixth day of the test. The neutralization titer of the serum was the highest dilution which protected the cell sheet from the cytopathic effect of the virus.

DISCUSSION

Any neutralization of BVDV in the one to four dilution or higher by a bovine serum was considered an indication that the animal had an infection with the virus or developed a passive immunity through the ingestion of colostrum. Passive immunity titers in calves of 1:4096 may be observed and it may take five months before a passive antibody titer of this magnitude will recede to negative at one to four.

The most reliable information from the serum neutralization test is obtained through the use of paired samples. The first serum sample should be collected as soon as possible after the disease is detected, preferably during the first six or seven days following the onset of signs. The second sample may be collected four weeks later. After an acute infection with the BVDV the peak antibody titer occurs in about five weeks. A sixteenfold rise in titer, say from 1:4 to 1:64, is considered a significant indication that the animal was infected with the virus. If the interval between the paired samples was only one or two weeks, a fourfold rise in titer may be equally valid.

Serums may be routinely screened at the one to four dilution to save time since a negative finding at this titer is good evidence that the animal from which the serum was collected had no contact with BVDV. After eliminating the negative serums in this manner, the serums having antibody titers may be tested in higher dilutions to determine endpoint titers.

Neutralization tests for IBRV may be interpreted in a similar manner with appropriate emphasis on ascending titers.

REFERENCES

1. Diagnostic Services, National Animal Disease Laboratory, Coverslip Tissue Culture, Fluorescent Antibody Technique for the Detection of Hog Cholera Virus. (Mimeograph)
Toxicology designates the science of poisons. It encompasses the effects of poisons on body functions, specific antidotes and treatment for these effects, the identification and quantification of these agents in body tissues as well as the interpretation of the data obtained from such analysis. In this instance, discussion will be limited to those poisons of primary importance in veterinary medicine. By definition, these compounds are substances which if introduced into or upon the animal body may produce death or serious injury. Usually, the word poison is reserved for compounds which produce their effects in relatively small doses. This may not always be the case. Sheep have been maintained on two percent solution of sodium chloride as their sole source of water for weeks, with the only measurable effect being some weight loss. On the other hand, there are many instances of death in animals following the ingestion of feed containing larger amounts of sodium chloride. The fatal dose of nicotine for a horse may be as little as 0.3 gram and for sheep even less. Nicotine sulfate has been widely used as an anthelmintic. Both sodium chloride and nicotine would be considered poisons. The dosage and animal sensitivity must then be criteria of a poison.

A horse came to my attention who died following convulsions brought on by strychnine given as a tonic. By accident, more than one groom had administered the prescribed dose. An autopsy revealed that the horse had broken a hip joint during the convolution, and the jagged edge of the bone had severed a large blood vessel. In this case a poison was administered in high enough concentration to cause the accident but not produce the death. Death resulted from a massive hemorrhage. Prior to this finding, charges of animal homicide were made implicating a rival horse owner. It was only after the post mortem examination that the accident was uncovered. The causes of most accidents will be determined if the investigation is adequate.

If, following such an investigation, analyses for poisons are indicated, it is desirable to collect and preserve the specimens and information that will give the analyst the best opportunity to find the poison or rule it out. Because of the many classes and large numbers of poisons, it is impractical to search for even a large fraction of them. The chemical analysis of tissue is very time consuming and requires expensive equipment. For these reasons the search must be limited as much as possible based on the circumstances and on the observations of the investigators.

It is important to know the interval of time which may have elapsed between the onset of symptoms in the animal or animals and in the
collection of materials for analysis. Such information may help establish the rate of absorption and the relative toxicity of the agent. If such body material was not collected soon enough, the poison may have disappeared or be present in low concentration in the body materials. Some poisons are eliminated or detoxified at rapid rates. Death or injury may result from the alteration in body functions produced by the poisons which persist after such poison has been eliminated. For this reason, materials should be collected for analysis as soon as possible after the advent of symptoms.

A careful record of the symptoms should be made. Vomiting, diarrhea or abdominal tenderness may be produced by a wide variety of poisons or toxic plants as well as disease. Heavy metals like arsenic, antimony, lead or mercury, corrosives like strong acids or bases, miscellaneous poisons like fluorides, chlorates, carbon tetrachloride and many plants will cause these symptoms. Convulsions may follow the ingestion of cyanides, nitrates and nitrites, lead, strychnine, camphor and plant toxins. Coma would be produced by drugs like bromides, barbiturates or agents like carbon monoxide, nicotine, turpentine and some solanaceous plants. The toxicologist would like to know, then, if the animal suffered incoordination, his respiratory rate, his heart rate, the size of his pupils, the odor of his breath, the color of his urine, which may be green if phenols have been ingested, brown or black with acorns, etc.

If the animal has vomited, the vomitus should be saved. This may be the best source of the poison in a relatively pure state. In such case its identification would be simplified. Stomach contents are likely to contain the largest quantity of the poison. If the poison is unaltered by the body, reactions to specific reagents will be more characteristic.

The material which is saved for chemical analysis, whether it be vomitus, urine or blood from a living animal or tissues taken at a post mortem examination, should be stored in clean containers. A separate container should be used for each specimen. A wide mouth jar is preferable because of the ease of putting specimens in and taking them out. The jar should have a lid. Fruit jars, either pint or quart size, make excellent choices.

If the case has any medicolegal implications, the lids should be sealed over the specimen with tape in such a way that they cannot be opened without obviously disturbing the tape.

The containers should be labeled with the name of the veterinarian, the date and the identities of both the specimen and the animal from which they came.

If the stomach contents are large, a true aliquot should be collected. This can be done by thoroughly mixing them before filling the jar. If all the contents are not preserved, the approximate weight of the total should be placed on the label. In this way, an estimate can be made of the total poison consumed.

A loop of intestine may be collected. In this case, it should be tied off before it is removed from the body.
Inside the gastro-intestinal tract is still outside the body as far as most poisons are concerned. The toxic agent must be identified in a body organ in order to prove absorption. It must be quantitated to establish its role in causing or contributing to the incident. The exception to this rule would be the case of corrosives in which very serious damage could result without any appreciable absorption of the agent from the stomach or intestine.

Care must be taken to insure that the containers are clean. The analyst must base his interpretation on what he finds, and he must assume that what he found came from the specimen. If the container is contaminated, the analysis may reveal quantities of the poison in line with those to be expected in chronic or even acute intoxications.

In most cases specimens of liver and kidney in addition to stomach contents will be adequate for a good toxicological analysis. If chronic arsenic poisoning is suspected, skin, hair and hoof should be collected. Arsenic is stored or excreted in these tissues. It may be found here some time after it has disappeared from liver and kidney or urine and feces. Lead and arsenic are stored in bone. Therefore, in some instances, bone should be collected.

Whatever specimens are chosen, adequate amounts of them should be saved. In many cases less than one milligram of the poison is present in 100 grams of tissue. The fatal dose of strychnine for a dog is less than one-half milligram per pound of body weight. The death of a cow has been reported following the ingestion of 33 milligrams of strychnine. For toxicological analyses 100 gram samples are preferred. No more than one-third of the total sample should be used for an analysis. If poison is found to be present, a second aliquot must be analyzed to corroborate the first analysis. A third aliquot must be preserved in the event it is desirable for a second laboratory to confirm the findings. Occasionally, an accident may result in the loss of the specimen so some must be saved for this eventuality. It is far easier to secure sufficient tissue at the post mortem than it is to obtain it later. The disposal of excess sample is easy. Ideally then, a toxicologist would like to have 500 grams of liver, kidney and stomach contents or, if this is not possible, all that is available. Ten grams of rib bone, skin, hooves or hair would be enough if these are to be sent in. Any errors in amounts should be made on the side of abundance of specimen.

When one has to work with less than optimum amounts of sample, the analysis becomes more difficult. Yields of the poison are less and there may not be enough purified extracts to make a proper number of tests. The greater the number of tests, the more certain the analyst can be of his results.

Any material submitted for chemical analysis for poisons should be preserved by freezing or refrigeration only. If any preservative is added, the tests are further complicated. Formaldehyde will destroy cyanides. It will fix tissues so that extraction of poison is more difficult. It will make addition products which may give reactions similar
to those given by the poisons being sought. The fresher the material the better the analysis will be. In decomposed tissue, compounds may be formed which interfere in the analysis. Pltoxines will be extracted with other basic organic poisons like strychnine and may give similar reactions. Under these circumstances, confirmation of strychnine will be more difficult.

When, in a case of suspected poisoning, a careful post mortem examination has been performed and the toxicologist has been supplied with adequate specimens, properly labeled, preserved, stored and delivered promptly together with a report of a thorough investigation and the gross autopsy findings, a thorough search can be made of the submitted material, and the chances of either finding a poison or ruling poison out are excellent. The analyst is then faced with the last and most important of his problems—the interpretation of the results.

Common metal poisons appear normally in animal tissue. Arsenic and lead are common contaminants of soil, feed and water; others like cadmium may be given therapeutically; still others like mercury are present accidentally.

Thus, while arsenic is not an essential element, it may be present in tissues from environmental exposure. An adult sheep can absorb over eighty milligrams of arsenic through the skin when dipped in a 0.20 percent solution of arsenic trioxide. If an open wound is present, even more would be absorbed. Fowler’s solution, which is one percent equivalent of arsenic trioxide, has been used to treat cattle. This would deposit amounts of arsenic in the tissues in excess of that to be found following average environmental exposure. In any event the concentration of arsenic, expressed as metallic arsenic, would rarely exceed 0.10 milligram per 100 grams of liver or kidney tissue unless symptoms of arsenic intoxication were present. The concentration of arsenic in muscle, fat or brain would, in every case, be less than in liver or kidney.

Arsenic is excreted in urine, milk and feces. If these materials, from any animal, were examined for arsenic, arsenic would be found to be present. Normal urine will contain up to 0.10 milligram of metallic arsenic per liter.

Analyses for arsenic, then, must reveal amounts in excess of these normal findings before the symptoms of staggering gait, abdominal pain, great thirst, salivation, vomiting, collapse and paralysis can be accounted for on this basis. In acute cases, death may follow within a few hours of the poison ingestion. If the exposure is sub-acute, the animals may live for days with loss of appetite, paralysis of the hind-quarters, stupor and sub-normal temperatures. Chronic exposure has occurred around smelters or where feed is excessively contaminated. In large animals this presupposed the ingestion of from one to three grams of arsenic daily over periods up to fourteen weeks.

Habitual exposure to excessive amounts of arsenic will not produce any degree of immunity although the gastro-intestinal tract may become increasingly resistant to absorbing arsenic at the usual rate.
Probably the most frequent cause of accidental poisoning in large animals is lead. Cattle are the usual victims. In most cases the source is lead paints containing oxides of lead, lead carbonate, lead chromate or lead sulfate. Lead arsenate, a tree spray, is a common offender. In this case, symptoms of arsenic poisoning would be superimposed on those due to lead. Lead acetate or sugar of lead is an astringent material and is widely used for irritations of the skin and mucous membranes. It has been used to treat diarrhea. Pastures have been contaminated by industry with lead containing dusts, but relatively few animals have been so poisoned.

Fortunately, most ingested lead is excreted never having been absorbed. Even with the most soluble compounds, only about ten percent of that consumed is absorbed. This is most fortunate because the elimination of absorbed lead is very inefficient. Excretion is largely through the kidney. From the circulation, it is taken up first by soft tissue and then more slowly deposited in bone in much the same way calcium is stored. Thus, the highest concentration will be found in the bone followed by the liver and kidney with the smallest amounts in the muscle and brain.

Following acute intoxication, however, the highest concentration of lead will be in the liver and kidney followed by the bone.

Excretion of lead is so slow that high blood and urine levels may be found months after an acute or chronic exposure.

Although older animals are less susceptible to lead poisoning than their young, an average fatal dose is about one-half gram per pound of body weight.

Animals are almost always acutely poisoned. Symptoms are usually delayed for 48 or more hours. These usually begin with staggering and frothing. This is followed by convulsions and an apparent blindness. The animals are in obvious distress with muscular spasms. Death may follow the onset of symptoms by a few hours. Death may be delayed for several days if the exposure was not as acute, during which the symptoms continue with either diarrhea or constipation. Feces from lead poisoned animals have been reported to have a distinctive odor. Horses exhibit less well defined symptoms but usually show stupor and some paralysis.

Lead inhibits a wide variety of body enzymes, and death results from such inhibition. A post mortem examination will reveal an inflammation of the abomasum and intestine in sheep but not always in cattle. Diffuse hemorrhages may be seen in the epicardium, the brain and the spinal cord. Degenerative changes will be apparent in the liver and other soft tissues.

The diagnosis can be made from the symptoms, the post mortem findings and the chemical findings. Chemical findings alone can rarely be depended upon. The central nervous system symptoms may be confused with those found in other disease entities. The lack of an apparent source of lead should not deter a search for old paint cans or flaking from painted wood. The flakes may not be found at autopsy.
The blood of animals not exposed to excessive lead will contain less than 0.10 milligram per 100 milliliters and usually around 0.05 mg/100 ml. In cases of excessive exposure this concentration will increase five to ten times. The concentration of lead in liver and kidney will exceed one milligrams per 100 grams of fresh tissue and may be two to five times this amount. With chemical data alone, death may be safely explained by lead when the kidney and liver have concentrations of two milligrams per 100 grams of fresh tissue or more.

The treatment of lead poisoning is fairly specific: with the use of a chelating agent, calcium versenate (EDTA). This compound will exchange calcium for lead and is then excreted as an inert chemical. It is administered intravenously at the rate of about 0.5 grams per 30 pounds of body weight per hour for two hours. The dose is repeated daily for at least five days. A rest period of several days will permit time for more lead to be mobilized from the bone to the soft tissue, and then, the treatment can be repeated. The versenates are relatively non-toxic. Over ninety-nine percent of a dose is eliminated within twenty-four hours. Toxic symptoms from overdoses are rare, and I know of no fatalities from this source.

Organic phosphorous compounds are being used in increasing amounts as potent insecticides. Their toxicity for animals depends on their action to inhibit cholinesterase. Cholinesterase is an enzyme which destroys acetylcholine, responsible for nerve impulse transmission. If the cholinesterase is inhibited, the animal suffers from prolonged stimulation of the nerves mediated by acetylcholine.

These compounds are readily absorbed by all routes. The common names of some of these compounds are parathion, dimeton, malathion and dipterex. All of these compounds show a cumulative effect. They are extremely toxic. Chronic exposure may result in pathologic changes unrelated to cholinesterase inhibition. For example, some of them produce demyelination of certain nerves resulting in different types of nerve paralysis. The symptoms due to cholinesterase inhibition result from excessive parasympathetic activity. These include profuse salivation, vomiting, diarrhea, and abdominal distress. Death is produced by respiratory failure, following convulsions of voluntary muscles. Excessive salivation and lacrymation with muscle twitch in the legs are the symptoms which first appear.

There are no primary lesions to be seen at autopsy. Diagnosis is made by a history of exposure, symptoms and a chemical analysis of whole blood for cholinesterase. Metabolites may be found in liver tissue. For analysis the toxicologist requires five milliliters of whole blood containing an anticoagulant like heparin. Sodium fluoride would interfere with the determination and should never be used.

The intoxication may be treated with atropine, which will abolish the muscarinic effects. In addition (PAM) 2-pyridine aldoxime methiodide is effective in reactivating cholinesterase.

Farm animals have been poisoned with nitrates and nitrites which contaminate water leaching manure piles or fertilized fields. The most
common sources, however, are plants grown in high nitrate soil which can take up enough of the salts to provide a toxic dose for animals eating them. One of the most common of such plants is oats, which produces "oat-hay poisoning;" over 50 varieties of weeds have been indicated with this potential hazard.

Nitrates depend for their toxicity on being converted to nitrites in the gastro-intestinal tract. Nitrite converts hemoglobin into methemoglobin, rendering it unable to transport oxygen. If 30 percent of the hemoglobin is so combined, symptoms of tissue anoxia will develop. Pigs are most susceptible to this type of intoxication. The minimum lethal dose of nitrite for them is less than 0.05 gram per pound of body weight. Poisoning has occurred in pigs eating large amounts of whey containing calcium nitrate as a preservative. Nitrites produce abdominal pains, diarrhea and cyanosis. Death can take place with little or no signs of clinical symptoms. At death the tissues are dark with the methemoglobin. Chemical analysis will reveal the high concentration of methemoglobin in the blood and nitrates and nitrites in the gastric contents.

Treatment depends on the reduction of methemoglobin to oxyhemoglobin. For this purpose methylene blue or ascorbic acid has been used. Intravenous methylene blue in doses of 10 milligrams per pound of body weight is effective and can be repeated if necessary. If oxygen is available, it would be a valuable adjunct.

Any discussion of toxicology and animals should include some comments about small animal pets. Toxicologists are frequently called upon to make analyses for poisons to explain the unexpected deaths of pets. Because facilities are usually so limited and the laboratory's primary responsibility is for people, such cases must carefully be selected. Because a poison analysis is lengthy and expensive, it can not be done merely to satisfy curiosity as to the cause of death. The only legitimate excuse for becoming involved depends on whether the analysis will assist in the prosecution of a pet poisoner. If so, any amount of effort is worthwhile. Of course, when the possibility exists that children might ingest poison bait put out for pets, everything possible must be done to identify the hazard to assist in the investigation.

It must be remembered that no matter how despicable or heartless the poisoner's act is, legal action is fruitless if there are no witnesses or evidence to support the charges. If the poison was eaten on the poisoner's property, the animal owner has little recourse.

The police should be expected to act in those cases when children have access to the baits. This is usually the case when several pets in a neighborhood are poisoned. We have had one incident of a child who was poisoned with cyanide from candy put out for dogs. Many times several pieces of poisoned meat have been picked up, and each one represented potential death for the consumer. The best disciplined animals, children or pets, can be expected to eat whatever appeals to them.
With the rapid advent of new economic poisons hazards to livestock and pets increase at a rate exceeded only by the problems of their toxicology. New analytical techniques are required. Information on the mechanism of action of these poisons is necessary if suitable treatment for the inevitable intoxications is to be developed. Such work can not be economically accomplished by isolated workers in remote institutions with little if any rapport. Multidisciplinary Institutes, financed by federal grants would provide the most optimistic approach to this problem. These institutes should be staffed by competent investigators who can coordinate their efforts in the interest of better veterinary medicine. This organization would not burden itself with routine toxicology or microbiology or pathology. Rather it would encourage the development of new techniques in all these areas for routine studies by others. Serving as a national resource it would develop basic information on the action of poisons at the cellular and subcellular level. Such an institute could clinically evaluate the hazards of such compounds in animals. This would not only benefit veterinary medicine, but would be a significant contribution to the national economy. Such an institute should include pathology. Valuable records could be maintained to keep the profession advised on current problems and their extent. Difficult and unusual cases could be submitted for extensive study and evaluation.

Every practicing doctor should have a toxicology facility available on a regional basis. Ideally these should be located at colleges of veterinary medicine. State laboratories can fill this need if they are adequately staffed and budgeted. The State Laboratory of Toxicology for Indiana is located at the Medical School and its primary function is in the area of human toxicology. It has provided services for animal toxicology and could do more if an effort were made to advise legislators of the need.

The problems are out-growing local laboratory support and must be met with a national effort. The development and expansion of regional facilities in boards of health and universities is essential but can not be enough. Such laboratories must be complimented with continuing toxicological basic research on new methods of analysis, mechanisms of action and of long range effects of poisons, interpretation of analytical data and treatment of poisoned animals.
The accurate diagnosis of poisoning in animals by organic pesticides requires thorough investigation, astute observation and the evaluation of various types of evidence. This paper is an attempt to relate the relative importance of field investigations, symptoms, lesions and chemical analyses in the diagnosis of pesticide toxicities. No single type of evidence should be used by itself to make a diagnosis, but when evaluated together and when proper significance has been placed on each, these four sources can usually provide the information necessary for making a proper diagnosis.

Frequently, cases of pesticide poisoning have legal implications. This requires that an investigation leading to a diagnosis be conducted in a legally acceptable manner: thorough, complete, and unbiased on the part of the investigator.

**FIELD INVESTIGATIONS**

Unlike many infectious diseases, poisoning by organic pesticides can rarely be accurately diagnosed from the laboratory. Field investigations are almost always necessary. Rarely will a chemical test, an animal showing signs, or a pathological examination of an animal submitted to a diagnostic laboratory provide sufficient evidence to make a positive diagnosis. Personal inspection of the premises should be made by the diagnostician, who should note the circumstances that may have contributed to poisoning, and inspect the feedlots, dipping vats, and spraying equipment. Hear-say or second hand evidence should not be relied upon, as such evidence frequently gives erroneous impressions and at best is not acceptable in a court of law. The investigator should develop a questioning attitude, insisting upon learning the details of management and care of the animals that may have resulted in poisoning.

Such facts as the ratio of the number of animals affected to the total number of animals in the unit, the date and time of losses, and symptoms observed should be obtained. An attempt should be made to determine all possible avenues of exposure to the toxicant, whether deliberate or unintentional. Additional information should be obtained, such as the name(s) of pesticide(s) involved, the concentration used, and how applied. Errors in the calculation of dosage or mistaken identity of a compound are common causes of poisoning. Sometimes

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plant formulations have been used on animals with disastrous results. Farmers have fed insecticides to livestock thinking them to be salt or a mineral supplement. The investigator should be prepared to calculate the probable exposure level if a specific pesticide is suspected. If the relative toxicity of a pesticide is known, and probable exposure level has been calculated, a reasonably good estimate of the probability of toxicity can be made. For example if a pesticide that is known to be toxic at a one percent concentration and is recommended for use at 0.5 percent, is mistakenly calculated and applied at a five percent concentration, the likelihood of poisoning is great. This is true because we have actually administered five times the toxic dose and ten times the recommended dose.

When miscalculations of dosage or other errors in administration are not obvious, predisposing factors may be involved, such as the physical condition of the animals, state of lactation, age, breed, accompanying stress, and weather conditions. Emaciated and lactating animals are several times some susceptible by such pesticides as BHC and toxaphene than are non-lactating animals in good condition. Young animals are generally more susceptible to poisoning than are adults, although there are notable exceptions, such as Ronnel which is equally toxic to adult and young cattle. Certain breeds are more susceptible than other breeds to specific pesticides. For example, brahman cattle are highly susceptible to poisoning by the organophosphorus compounds, Ciodrin and Compound 4072. It is generally thought that subjecting an animal to stress during or immediately following exposure to a pesticide may increase the likelihood of poisoning. The opposite of this has been reported, however, in the case of the organophosphorus compound trichlorfon in that placing an animal under stress will decrease the effects of a toxic dose of this compound. Practices such as castration, vaccination, and dehorning performed at the time of pesticide exposure, especially during hot or cold weather, may also result in increased likelihood of poisoning. Certain therapeutic agents may potentiate the toxicity of some pesticides. Classic examples of such potentiation are phenothiazine compounds and high levels of vitamin A, which may increase the toxicity of the organophosphorus pesticides, Coumaphos and Trichlorfon.

An important part of the field investigation is the determination of the signs displayed by the affected animals. A complete account of all signs should be obtained with particular attention being given to the details of their description and the progression of events. Although not pathognomonic for a specific pesticide, a complete account of symptoms displayed can aid in making the diagnosis.

The type and amount of drug therapy used and the responses to such therapy may aid in the differentiation of pesticides from each other as well as from other toxicities, and from infectious or nutritional diseases.

Most of the synthetic organic pesticides act rapidly, usually within 24 to 48 hours. The appearance of new cases appearing on succeeding
days is an indication that an infectious agent may be involved. The pattern of symptoms should be compatible with the published descriptions of symptoms for the various pesticides.\textsuperscript{1,2} Many infectious diseases produce signs indistinguishable from those described for certain insecticides. Rabies, listeriosis, and thrombo-embolic meningoencephalitis may produce central nervous system (CNS) signs similar to those produced by chlorinated hydrocarbon insecticides. Conditions such as polioencephalomalacia, salt poisoning, plant and other inorganic toxicities, neoplasias, and invasions by parasites may be manifested by signs similar to those produced by pesticides.

Finally, the information obtained during the field investigation should be assembled and the facts sorted and organized. Usually a hypothesis can be formulated regarding the probability of pesticide poisoning if a good investigation has been conducted.

SYMPTOMS

The symptoms associated with organic pesticide poisoning are not pathognomonic. However, if one is familiar with the symptoms as well as the characteristic sequence of events usually associated with pesticide toxicity, it will greatly aid in making the diagnosis. Signs of organic pesticide poisoning vary with the compound, the species of animal involved, and to a certain extent the route of administration, whether topical, by inhalation, ingestion or parenteral.

The organophosphorus compounds are inhibitors of the enzyme cholinesterase, which normally inactivates the acetylcholine produced at neuro- and myo-neuro junctions. A small excess of acetylcholine at a junction causes an abnormal increase in function, whereas a large excess produces an abnormal decrease in function, and symptoms may vary accordingly. Two types of cholinesterase enzymes have been recognized in most animals. One type is true cholinesterase which has a specificity for acetylcholine and is found in the nervous system, muscles, glands and erythrocytes. Inhibition of this enzyme is the most important of the two in the production of signs of toxicity. A second, non-specific or pseudo-cholinesterase, is found in plasma, various tissues, and the nervous system, and is capable of inactivating a wide range of esters including acetylcholine. It is important to keep in mind that the cholinesterase found in the whole blood of livestock is generally not involved in the appearance of signs of poisoning due to cholinesterase depletion. For this reason the whole blood cholinesterase determination is not a diagnostic test. Depletion of this cholinesterase may not correlate with the degree of depletion in the nervous system and other tissues. This is why an animal may show signs of poisoning with only a slight depression of blood cholinesterase or may show no signs of poisoning with complete depletion of blood cholinesterase. If the depletion of the blood cholinesterase level happens to reflect the depletion of true cholinesterase at the nerve endings, as is the case with some organophosphorus compounds, it then has diagnostic significance.
Values for normal animals vary considerably, however, and unless the pre-exposure level of blood cholinesterase is known, little significance can be given to low values after exposure to pesticides.

In general, signs of organophosphorus poisoning are those of over-stimulation of the parasympathetic nervous system. These include: profuse salivation; gastrointestinal hypermotility resulting in severe pain, abdominal cramps, vomiting, and diarrhea; excessive lacrimation; sweating; dyspnea with rales; miosis; pallor; cyanosis; and incontinence of urine and feces. Excessive stimulation of the skeletal muscles may occur and is manifested by twitching of the muscles of the face, eyelids, tongue, and ultimately the general musculature. This hyperactivity is followed by weakness and paralysis of the skeletal muscles. Stimulation of the central nervous system followed by depression may occur. Death results from respiratory interference which may be due to bronchial constriction, paralysis of the respiratory center and/or excessive accumulation of fluid in the lungs. The organophosphorus compounds also cause cardiac malfunctions resulting in bradycardia and heart blockage. In general, atropine has the ability to counteract these symptoms in domestic animals. The accompanying table lists the more common pesticides plus toxicity data and symptoms in livestock.

The carbamate compounds, of which physostigmine is a well known naturally occurring example, are also cholinesterase inhibitors. Signs of carbamate toxicity are usually similar to, yet sometimes distinctly different, from those produced by the organophosphorus compounds in that they usually are more exaggerated. Signs appear within a few hours after exposure and the course of the clinical effect is rapid. Atropine is also an effective antidote against carbamates. (See table.)

Those pesticides which may be translocated throughout the treated plant or animal in amounts sufficient to kill pests feeding in or on the tissues or fluids are referred to as having systemic activity. All such systemic pesticides presently in use are classified as organophosphorus compounds. Other types of chemicals possess systemic activity, but have been discarded for various reasons, not the least of which is the resulting high residue levels in the tissues. Although included as organophosphorus compounds, the systemic pesticides cannot clearly be classed as such because they possess other chemical moieties which produce different effects, mainly those of chlorinated hydrocarbon pesticides. Signs of poisoning produced by these pesticides may be those of either organophosphorus compounds or chlorinated hydrocarbon compounds, or both. Atropine may have only limited antidotal effect. (See table.)

The chlorinated hydrocarbon compounds produce CNS stimulation or depression usually associated with neuro-muscular manifestations. Two different types of syndromes have been recognized, (1) those caused by the DDT group, and (2) those caused by the cyclodiene group (BHC, Chlordane, Aldrin, Heptachlor, Toxaphene, etc.).

Symptoms of the DDT group are initially manifested by abnormal fear, and violent responses to subthreshold stimuli which normally do
not produce a clinical response. The animals are hypersensitive and have an increased frequency of spontaneous movements. As symptoms increase, hyperirritability similar to that seen in strychnine poisoning develops. Convulsions do not appear at this time, but tremors and finally tonic-clonic epileptiform convulsions may be seen as poisoning increases. All signs are strengthened by external stimuli. No abnormal positioning or labyrinth reflexes occur.

Symptoms of toxicity produced by the cyclodiene group include either CNS stimulation or depression. It is unlikely that a single animal will show all the possible signs, but there is usually enough similarity between animals that a definite syndrome can be recognized. All species of livestock and poultry show similar signs of toxicity if the wings of poultry are compared to the front limbs of livestock. Initially the affected animal may show unusual alertness and excitability, followed by twitching and spasms of the muscles of the face and neck. The onset of signs may occur within a few minutes lasting for a brief period of time; or may take two to three days to appear and may persist for several days, depending upon the pesticide and the dosage involved. There may be an explosive reaction during which the animal progresses through the early stages into clonic-tonic seizures very suddenly; or the symptoms may steadily progress in severity and terminate in convulsive seizures.

Frequently, affected livestock will alternate between convulsions and periods of severe depression, becoming completely oblivious to their surroundings. Abnormal posturing, such as standing with the head down between the front legs, resting the sternum on the ground with the rear part of the body standing, or standing and pushing against solid objects are frequently observed. Occasionally, an affected animal will show belligerence or may show continuous chewing movements. During convulsions the body temperature may reach 113-116°F, and peculiar outcries may be made. There may be a secretion of thick, copious saliva. Some affected animals may show no neuro-muscular symptoms, but become depressed and refuse to eat, drink, or respond to stimuli. They may remain in this condition for several days, then recover or suddenly terminate in convulsions and death.

The severity of symptoms displayed is not a good index of the animal's chances for survival. Some may die following a single convulsive seizure while others may survive repeated seizures and even several hours of apparent coma.

LESIONS

The lesions associated with the organophosphorus and carbamate compounds are not specific. Cyanosis, pulmonary edema, hemorrhages on the surface of thoracic and abdominal viscera, and congestion of the internal organs may be present.

Likewise, the lesions produced by the chlorinated hydrocarbon compounds are not specific and may be absent. Cloudy swelling of the
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Animal</th>
<th>Age</th>
<th>Maximum Dose Tested (Mg/Kg)</th>
<th>Minimum Dose Found (Mg/Kg)</th>
<th>Maximum Dose Tested (%)</th>
<th>Minimum Dose Found (%)</th>
<th>Symptoms of Toxicity and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORGANOPHOSPHORUS COMPOUNDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbophenothion</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.05</td>
<td>Typical symptoms of organophosphorus compounds; dyspnea, salivation, stiff movements, incoordination, collapse and death. No significant lesions.</td>
</tr>
<tr>
<td>(Trithion, Stauffer R-1303)</td>
<td>Cattle</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>0.1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>10</td>
<td>25</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Ciodrin (Shell 4292)</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>---</td>
<td>---</td>
<td>0.5</td>
<td>2.0</td>
<td>Brahman cattle are especially susceptible; toxicity has occurred when 0.1% has been applied dermally. Typical symptoms of organophosphorus compounds; muscular weakness and diarrhea are pronounced. No significant lesions.</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>2.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>1.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>1.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>3 mos.</td>
<td>---</td>
<td>---</td>
<td>2.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Compound 4072 (2-Chloro-1,2,4-trichlorophenyl vinyl diethyl phosphate)</td>
<td>Cattle</td>
<td>all ages</td>
<td>---</td>
<td>20</td>
<td>---</td>
<td>0.15</td>
<td>Brahman cattle are more susceptible to toxicity. Symptoms: excessive salivation, dyspnea, weakness, diarrhea and collapse; muscular stiffness is usually absent. No significant lesions.</td>
</tr>
<tr>
<td>Dichlorvos (DDVP, Vapona)</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>---</td>
<td>10</td>
<td>1% dust-2 oz., total</td>
<td>---</td>
<td>Very rapidly metabolized; course may be a few minutes or a few hours. Symptoms: dyspnea, excessive salivation, stiffness; horses may exhibit colic and diarrhea. No significant lesions.</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>2% mist-200 ml total</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>adult</td>
<td>---</td>
<td>25</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>---</td>
<td>25</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>0.5</td>
<td>2.5</td>
<td>0.05</td>
<td>0.1</td>
<td>Data given pertain to wettable powder only, emulsifiable materials tend to become more toxic with age. The reaction of ruminants is highly variable. Symptoms: dyspnea, excessive salivation, stiffness, diarrhea and tongue held extended in cattle. Course may be from 1 to 24 hours. No significant lesions.</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>6-12 mos</td>
<td>10</td>
<td>25</td>
<td>0.25</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>20</td>
<td>30</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>adult</td>
<td>20</td>
<td>30</td>
<td>---</td>
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</tr>
<tr>
<td></td>
<td>Horse</td>
<td>adult</td>
<td>20</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>adult</td>
<td>---</td>
<td>2</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Dioxathion (Delnav)</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>---</td>
<td>5</td>
<td>---</td>
<td>0.1</td>
<td>Rapidly eliminated from the body. Course runs from a few minutes to a few hours. Symptoms: excessive salivation, dyspnea, stiffness, followed by weakness, prostration and death. Lesions: generalized cyanosis and pulmonary edema.</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>0.5</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>---</td>
<td>0.5</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>adult</td>
<td>---</td>
<td>0.25</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.25</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Systemic Organophosphorus Compounds</td>
<td>Adult cattle and calves are equally susceptible. Symptoms: excessive salivation, depression and mild stiffness. Animal may stand with arched back and evidence of abdominal pain. No dyspnea. No lesions.</td>
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</tr>
<tr>
<td>Ethion (Nialate, Niagara 1240)</td>
<td>Calves: 1-2 wks --- --- 0.25 0.5 Sheep: adult --- 25 0.5 1.0 (lethal) Goat: adult --- --- 0.25 0.5</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Famophos (American Cyanamid 38023)</td>
<td>Calves: 1-2 wks --- 10 &gt;10 --- --- Sheep: adult 50 &gt;50 --- --- Goat: adult 50 100 --- --- Horse: adult 50 --- --- ---</td>
<td></td>
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</tr>
<tr>
<td>Guthion (Bayer 17147)</td>
<td>Calves: 1-2 wks --- 0.1 0.5 Sheep: adult 12 25 --- ---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion (Compound 4049)</td>
<td>Calves: 1-2 wks --- 10 20 --- 0.5 1.0 Sheep: adult 50 100 --- 2.0 --- Goat: adult 50 100 1.0 1.0 Horse: adult --- --- 2.0 ---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl Trithion</td>
<td>Calves: 1-2 wks --- --- --- 0.5 --- Sheep: adult 25 --- --- --- Goat: adult --- --- 0.1 ---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td>Calves: 1-2 wks --- 0.5 --- --- 0.01 Sheep: adult --- 50 --- 1.0 --- Goat: adult --- 20 (lethal) --- 1.0 --- Swine: adult --- 25 (severe) --- ---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphamidon (Dimecron)</td>
<td>Calves: 1-2 wks --- --- --- --- Sheep: adult 5 --- 0.25 0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC 13 (0-2,4-dichlorophenyl-0,0-diethyl phosphoro-thioate)</td>
<td>Calves: 1-2 wks --- --- --- 0.25 0.5 Sheep: adult --- --- 2.0 3.0 Goat: adult --- --- 0.25 0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Very slow course, one day to several days. Chemicals such as pyrethrins, rotenone, (continued on next page)
TABLE I (continued)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Animal</th>
<th>Age</th>
<th>Oral</th>
<th>Dermal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maxmum Non-Toxic Dose Tested</td>
<td>Minimum Toxic Dose Found</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mg/Kg Body wt.</td>
<td>%</td>
</tr>
</tbody>
</table>
| Co-Ral, Asuntol, Muscatox)| Sheep  | adult   | ---     | 8     | 0.25     | 0.5    | phenothiazine, and high levels of Vitamin A. increase its toxicity. Other stress factors may increase toxicity. Symptoms: saliva
tion dyspnea, stiffness within 30 hours; dehydratation terminal stage; respiratory failure. Lesions: variable; lung congestion and edema, hemorrhages on heart; cyanosis. |
| Dimethoate                | Calves | 1-2 wks | ---     | 5     | 1.0      | ---    | Rapidly absorbed and metabolized. Symptoms: excessive salivation, dyspnea, abdominal discomfort, stiff limbs. Lesions not specific. |
| Fenthion                  | Calves | 1-2 wks | ---     | 50    | 0.25     | ---    | Symptoms typical for organophosphorus compounds. |
|                           | Cattle | 1 yr.   | 10     | 15    | 1.0      | ---    | |
|                           | Sheep  | adult   | ---     | 50    | 1.0      | ---    | |
|                           | Horse  | adult   | 50     | 60    | ---      | ---    | |
|                           | Goats  |         | ---     | 25    | ---      | ---    | |
|                           | Horses | adult   | 20     | 25    | ---      | ---    | |
|                           |        |         | ---     | 50 (lethal) | 0.5   | ---    | |
|                           |        |         | ---     | -     | ---      | ---    | |
| Imidan                    | Calves | 1-2 wks | ---     | 25    | 0.5      | 1.0    | Symptoms typical for organophosphorus compounds. |
|                           | Cattle | adult   | 10     | 25    | ---      | ---    | |
|                           | Sheep  |         | ---     | 50    | ---      | ---    | |
|                           | Goats  |         | ---     | -     | ---      | ---    | |
|                           | Horses | adult   | 20     | ---   | ---      | ---    | |
|                           |        |         | ---     | 25    | ---      | ---    | |
| Ruelene                   | Calves | 1-2 wks | 100    | 125   | ---      | ---    | Symptoms highly variable and unique. In some cases excessive salivation and colic; in others alternating depression and excitement; in others paralysis of limbs and body, but head and neck swings from side to side. Eye movements uncoordinated. Many animals go down on side and paddle with legs. |
|                           | Cattle | adult   | 100    | 125   | 2.5      | ---    | Slowly metabolized, toxic effects may linger 2-3 months. Symptoms: muscular weakness (drag hind feet) diarrhea within first 24 hours. With very high doses, excessive salivation, dyspnea, loss of weight and hair luster. Lesions not significant. |
|                           | Sheep  | adult   | ---    | 400   | 2.5      | ---    | |
|                           | Horse  | adult   | 110    | -     | ---      | ---    | |
|                           |        |         | 25     | 50    | ---      | ---    | |
| Ruelene                   |        |         | 25     | 50    | 1.5      | 2.0    | |
|                           |        |         | 100    | 125   | 2.5      | ---    | |
|                           |        |         | 150    | 200   | 5.0      | ---    | |
|                           |        |         | 150    | 100   | 5.0      | ---    | |
|                           |        |         | 25     | 50    | 2.5      | ---    | |
|                           |        |         | 50     | 75    | 1.0      | ---    | |
|                           |        |         | 100    | 100   | ---      | ---    | |
|                           |        |         | 100    | 100   | ---      | ---    | |
|                           | Cattle | adult   | 50     | 75    | 2.0      | ---    | |
|                           | Sheep  |         | ---    | 100   | ---      | ---    | |
|                           | Horse  |         | ---    | 100   | ---      | ---    | |
|                           |        |         | 50     | 75    | 1.0      | ---    | Domestic animals show more toxicity when on fresh green forage. Placing animal under stress will decrease the toxic effects. Rapidly eliminated from the body; rapid course, within hours. Symptoms: typical of organophosphorus compounds. Horses and sometimes cattle exhibit colic. No significant lesions. |
## Carbamates

**Carbaryl**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves</td>
<td>1-2 wks</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Cattle</td>
<td>adult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>adult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>adult</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Toxic doses for livestock have not been established.

**Pyroban**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves</td>
<td>1-2 wks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>adult</td>
<td>0.05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Onset of symptoms very rapid. Very similar to those of organophosphorus poisoning except each movement is greatly exaggerated. Animals may appear in a rage, stumbling and running into objects. Excessive salivation and dyspnea.

## Chlorinated Hydrocarbons

### Cyclodiene Group

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Cattle</td>
<td>adult</td>
<td>35</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>3 wks</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>3 wks</td>
<td>35</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Symptoms of cyclodiene group; hyper-sensitivity, apprehensive or belligerent. Muscle twitching about the face and neck followed by clonic spasm of cervical muscles, then the fore quarters and finally the hind quarters. Clonic-tonic convulsions may be intermittent, alternating with periods of depression; chewing movements; incoordination; aimless walking; abnormal posture; nystagmus; grinding of teeth. Symptoms may be progressive or explosive. Body temperature may reach 114°-116°F.

### Dieldrin

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dieldrin</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Cattle</td>
<td>adult</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Lambs</td>
<td>2 wks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>adult</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horses</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td>8 wks</td>
<td>25</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Death due to respiratory failure. Some animals show only marked depression and apneasitv. Lesions are non-specific, cloudy swelling of viscera with petechial hemorrhages, blanching of intestines; dark congested lungs; congested vessels in the brain with increased CSF pressure.

### Heptachlor

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptachlor</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Cattle</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>adult</td>
<td>25</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Heptachlor has highly cumulative effect. Symptoms of cyclodiene group.

### Lindane

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindane</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Cattle</td>
<td>adult</td>
<td>15</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambs</td>
<td>3 wks</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horses</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Symptoms of cyclodiene group. Especially toxic to young and emaciated animals particularly during lactation.

### Telodrin

<table>
<thead>
<tr>
<th>Carbamate</th>
<th>Animal</th>
<th>Age</th>
<th>Toxic Dose (mg/kg)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telodrin</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>0.01</td>
<td>0.025</td>
</tr>
<tr>
<td>Cattle</td>
<td>adult</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Symptoms of cyclodiene group.
TABLE I (Continued)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Animal</th>
<th>Age</th>
<th>Oral Maximum Non-Toxic Dose Tested Mg/Kg Body wt.</th>
<th>Oral Minimum Toxic Dose Found Mg/Kg Body wt.</th>
<th>Dermal Maximum Non-Toxic Dose Tested %</th>
<th>Dermal Minimum Toxic Dose Found %</th>
<th>Symptoms of Toxicity and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose Tested</td>
<td>Found</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Toxaphene</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>5.0</td>
<td>10.0</td>
<td>0.50</td>
<td>0.75</td>
<td>Emanicated, lactating animals more susceptible. Symptoms of cyclodiene group.</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>4 mos.</td>
<td>---</td>
<td>---</td>
<td>1.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>adult</td>
<td>25</td>
<td>35</td>
<td>2.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>10</td>
<td>25</td>
<td>1.5</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>1.5</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pig</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horse</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>1.5</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>(DDT Group)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>100</td>
<td>250</td>
<td>8.0</td>
<td>---</td>
<td>Cats are especially susceptible to DDT toxicity. They show extreme rigidity.</td>
</tr>
<tr>
<td>(Chlorophenothane, Gesarol, Neocid, dicophane)</td>
<td>Cattle</td>
<td>adult</td>
<td>250</td>
<td>500</td>
<td>8.0</td>
<td>---</td>
<td>Poisoning rarely seen in livestock by this group. Symptoms: hypersensitivity, increased spontaneous movements, tremor, followed by tonic-clonic convulsions.</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>250</td>
<td>500</td>
<td>8.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>adult</td>
<td>250</td>
<td>---</td>
<td>8.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>250</td>
<td>500</td>
<td>8.0</td>
<td>---</td>
<td>About half as toxic as DDT to livestock.</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>8.0</td>
<td>---</td>
<td>Symptoms of DDT.</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>1000</td>
<td>---</td>
<td>8.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>TDE</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>100</td>
<td>250</td>
<td>8.0</td>
<td>---</td>
<td>Dogs and chickens are susceptible to TDE toxicity. Symptoms of DDT.</td>
</tr>
<tr>
<td>(Rhothane, DDD)</td>
<td>Sheep</td>
<td>1-2 wks</td>
<td>---</td>
<td>---</td>
<td>8.0</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>adult</td>
<td>1000</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Perthane</td>
<td>Calves</td>
<td>1-2 wks</td>
<td>250</td>
<td>500</td>
<td>8.0</td>
<td>---</td>
<td>Symptoms of DDT</td>
</tr>
<tr>
<td>(Q137)</td>
<td>Sheep</td>
<td>adult</td>
<td>1000</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

*All data pertains to normal, healthy, non-lactating animals.
Data taken primarily from Radeleff and Radeleff and Bushland.
viscera with blanching of the intestines may be present if the animal had a high body temperature. Small hemorrhages throughout the body, especially on the heart and lungs may be present. The lungs are usually heavily congested and dark colored. The brain and spinal cord are also usually congested and may contain an excess of fluid causing an elevated cerebrospinal fluid pressure.

In chronic cases of chlorinated hydrocarbon poisoning non-specific degenerative changes in the liver and kidneys may be present. The animals usually lose weight and become dehydrated. In dogs poisoned by TDE, a severe atrophy of the adrenal cortex resulting in adrenal cortical insufficiency has been seen.

CHEMICAL ANALYSES

Chemical analyses should not be solely relied upon for making a diagnosis of organic pesticide poisoning. At best, only information concerning the nature of exposure and the identification of the compound can be obtained. Chemical residues on the hair and skin or in stomach contents may provide valuable diagnostic information, but such data should be evaluated along with the history, symptoms and the pathological examination before a diagnosis is declared.

Substantial residues of several pesticides may be present in body tissues, depending upon the exposure of the animals, without producing poisoning. This is true in many cases of chlorinated hydrocarbon compounds where such residues may be present while the actual toxicant is not. With other compounds, such as the rapidly metabolized organophosphorus compounds, tissue residues are not present even when the animal has been exposed to toxic levels.

When obtaining specimens for chemical analysis, the utmost care should be taken to assure that uncontaminated samples are submitted to the laboratory. The chemist is dealing with microgram quantities or less, and the slightest contamination may invalidate the test. Several types of specimens should be submitted including body fat, skin and hair, liver, kidney, brain, and rumen or stomach contents.

SUMMARY

Rarely can the accurate diagnosis of organic pesticide poisoning be accomplished without the evaluation of evidence obtained by field investigation and close observation of signs. Post mortem examinations and chemical analyses may provide corroborative evidence and aid in the differentiation of pesticide toxicities from other conditions.

REFERENCES

DIFFERENTIATION OF HEMATOZOAN PARASITES OF DOGS

S. A. Ewing

Differentiating the various helminth and protozoan parasites which occur in the blood of dogs is relatively easy given a representative sample and a good microscope. It is axiomatic that properly prepared specimens are essential if accurate determinations for diagnoses, based upon morphologic characteristics of parasites, are to be made; the preferred techniques are simple and will be mentioned but not discussed. Suitable methods for identification of species of *Dirofilaria*, *Haemobartonella*, *Babesia*, *Ehrlichia*, *Hepatozoon*, and *Histoplasma* in peripheral blood are included.

**Microfilariae:** Blood is examined using a modified Knott's technique in which one ml. of fresh whole blood (heparinized) is mixed with 10 ml. of two percent formalin and is centrifuged. Supernatant is discarded and sediment is stained with an equal portion of 1:1000 solution methylene blue. The microfilariae stain a medium blue and stand out against a light blue background.

Prior to 1956 the presence of microfilariae in the peripheral blood of dogs in the United States was considered conclusive evidence of *Dirofilaria immitis* infection. At that time Newton and Wright showed that not all canine microfilariae were *Dirofilaria*; rather, *Dipetalonema reconditum* was proved to be a common parasite of dogs, and a means of distinguishing the microfilariae was detailed. It is necessary to use a calibrated ocular micrometer to differentiate the two microfilariae; *Dirofilaria* larvae are 307-322μ long x 6.7-7.1μ wide while *Dipetalonema* microfilariae are 296-283μ long and 4.3-4.8μ wide. The latter also possess a so-called button-hook tail that is not considered a reliable differential characteristic. Sawyer, et al., have emphasized that only fresh, properly fixed blood samples may be used; microfilariae in blood which has been stored may undergo morphological changes which preclude accurate identification.

It is important to have a straight specimen to measure. Figure 1 depicts a properly fixed and stained specimen; microfilariae represented by Figure 2 are curved too much to be measured accurately with an ocular micrometer.

Blood films stained with Romanowsky stains generally are satisfactory for examination of the organisms discussed subsequently. Thick films are preferred by some workers in searching for parasites in cases of low parasitemia. I personally use a thin film, for experience has shown that the morphology is always clearer, i.e., parasites not obscured by overlying host cells.

**Haemobartonellosis:** *Haemobartonella canis* is rarely seen in...
HEMATOZOAN PARASITES OF DOGS

Figure 1. Microfilaria properly fixed and stained for measurement.

Figure 2. Microfilariae which are too curved to permit accurate measurement with an ocular micrometer.

abundance in the blood of intact animals; if an infected animal is splenectomyed, however, significant parasitemia usually will develop, sometimes with 90 percent of the erythrocytes parasitized. The morphology, as interpreted by light microscopy, varies from single coccoid forms to long chains which sometimes even appear to branch.¹,¹² A so-called "violin
Figure 3. *Haemobartonella canis* on erythrocytes. Notice the single coccoid forms, the chains, and the so-called violin-bow form.

Figure 4. Crescent or semi-lunar body; these enigmatic structures often are seen in the peripheral blood of anemic dogs. The "bow" form can also be seen. Figure 3 is a photomicrograph in which the various morphologic types are represented.

So-called crescent or semi-lunar bodies of unknown significance often are seen in anemic dogs, Figure 4. Occasionally these crescents can be seen to be parasitized by *Haemobartonella* as in Figures 5 and 6.
Babesiosis: Babesia canis was first reported in the United States in 1934 and for many years was thought to be restricted to the southern part of the United States. In recent years, however, babesiosis has been found in the Southwest, Middlewest, and East. Characteristic trophozoites occur in erythrocytes and stain well with Romanowsky stains;
a single host cell may contain from one to 16 trophozoites, Figures 7-12. The great majority of cells contain one, two, or exponential multiples of two, and this has led to the conclusion that the parasite reproduces, in the erythrocyte at least, by binary fission. When odd numbers of trophozoites are found, it is often obvious that binary fission is in progress, Figure 13. Erythrophagocytosis is common in dogs with babesiosis, Figure 14, and may be responsible in part for the anemia.
Figure 9. Erythrocyte containing two *Babesia canis* trophozoites. Notice similarity to *Plasmodium* sp. trophozoites, often called "ring" forms.

Figure 10. Erythrocyte containing four *Babesia canis* trophozoites.

which develops. Extensive erythrophagocytosis may be a valuable clue to aid in diagnosis.

*Ehrlichiosis*: *Ehrlichia canis* was first reported in the United States rather recently.\(^7\)\(^8\) The so-called mulberry or morula form which occurs in leukocytes of peripheral blood is the classical form of this parasite, Figures 15-17. These are sometimes seen to disperse within the
Figure 11. Erythrocyte containing eight *Babesia canis* trophozoites.

Figure 12. Erythrocyte containing sixteen *Babesia canis* trophozoites.
Figure 13. Erythrocyte containing three *Babesia canis* trophozoites. Notice that one parasite is much larger than the other two and is about to complete binary fission.

Figure 14. Monocyte containing phagocytized erythrocyte.
cytoplasm, Figure 18. In Old World literature\textsuperscript{3,4,13} it is stated that the morulae are found more commonly in monocytes than any other cells, but in the United States they have been seen in lymphocytes, monocytes, and neutrophils in that order of frequency. Infected leukocytes are recovered more easily in impression smears of lung than in peripheral blood,
Figure 17. A typical morula of *Ehrlichia canis*; individual units of the mulberry are distinguishable.

Figure 18. *Ehrlichia canis* morula in process of dispersing.

Figure 19. Other inclusions seen in leukocytes of the peripheral blood of dogs suffering from ehrlichiosis include the so-called initial body, Figure 20, and unidentified inclusions in neutrophils, Figure 21. The latter appear slate-gray on Wright's stained preparations.

The life cycle of *Ehrlichia canis* within leukocytes was described by French workers as a succession of morula, disintegrating morula,
Figure 19. Two leukocytes each containing an *Ehrlichia canis* morula—from an impression smear of lung.

Figure 20. Monocyte containing an initial body of *Ehrlichia canis*.

elementary body, initial body, morula; this sequence has not been confirmed in the United States.

Hepatozoonosis: *Hepatozoon canis* can be identified using peripheral blood smears, the gametocyte stage occurring in neutrophils and monocytes, Figures 22 and 23. This parasite has not been proved to occur in dogs in North America, but *Ehrlichia* was recognized here only three
Figure 21. Neutrophils containing small inclusions of unknown significance that appear slate-gray on Wright's stained preparations.

Figure 22. Neutrophil containing one *Hepatozoon canis* gametocyte.
years ago which prompts reservations in stating our problem. The schizogonous phase of the *H. canis* cycle occurs in the spleen, bone marrow, and liver, but confirmatory diagnosis is usually based upon gametocytes in circulating leukocytes.

*Histoplasmosis:* *Histoplasma capsulatum* has no rightful place in a
discuss the organisms, but since the presence of such organisms in peripheral blood may lead to confusion, they are included.6,22 Sometimes the organisms are seen in the cytoplasm, Figure 24, but they may be in the nucleus, Figure 25, or in both, Figure 26. The staining properties of this organism with Romanowsky stains are not as predictable as those of the hematozoans discussed.
REFERENCES

Sanitary measures, including formaldehyde-gas fumigation, have been effective aids to reduce the spread of disease producing organisms among poultry, however these measures alone have not been successful in preventing transmission of *Paracolon arizona*. Trials were conducted to determine if vaccination of turkey breeders would produce immunity to *P. arizona* and in addition transmit parental immunity to poults. These trials involving immunization of breeders with *Paracolon arizona* (type 7:1,7,8) bacterins have indicated that vaccination will improve control of *P. arizona* infection in turkeys.

TRIAL 1

A trial was conducted to determine if the vaccination of turkey breeder hens would offer any protection against *Paracolon arizona* infection of their progeny. Two different *P. arizona* bacterins were used in the trial. One flock of hens was inoculated intramuscularly with a commercially prepared product which was essentially a formalized whole broth culture of *P. arizona* with an aluminum hydroxide adjuvant. A second flock of hens was inoculated subcutaneously with a bacterin prepared from an agar grown culture of *P. arizona* with an oil emulsion adjuvant. These two flocks were vaccinated at 28 weeks of age, just before the beginning of egg production, and revaccinated six weeks later. The remainder of the flocks on this ranch were not vaccinated. All of these flocks were considered to have had natural exposure to *P. arizona* as poults.

Egg production for the flock that received the broth-type commercial bacterin was on a par with unvaccinated flocks and considered normal. Egg production for the flock that received the oil emulsion bacterin was considerably less than unvaccinated flocks and estimated to be 50 percent of normal.

Dead embryos and day-old poults from all flocks were submitted to the laboratory throughout the laying season. No isolations of *P. arizona* were obtained from eggs or poults of vaccinated hens. Several isolations of *P. arizona* were obtained from both eggs and poults of unvaccinated hens. Husbandry of all flocks included above average disease control measures.

Near the end of the laying season, six hens from each vaccinated flock, plus six hens from an unvaccinated flock, were removed to an isolated area for challenge. Each hen was given 15 mls of a 24-hour-broth culture of *P. arizona* by oral inoculation. Prior to its use for challenge,
TABLE I

*Paracolon Arizona* Isolations from Vent Swabs of Orally Challenged Hens

<table>
<thead>
<tr>
<th>Days after challenge when individual vent swabs were collected</th>
<th>Vaccinated Groups</th>
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<tr>
<td></td>
<td>Unvaccinated Group</td>
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<tr>
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<tr>
<td>Total</td>
<td>35</td>
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</table>

the isolate had been passed through turkey embryos to maintain virulence. Vent swabs of each hen were cultured on the day of challenge and on 12 occasions during a 25-day period following challenge. Table I records the number of times *P. arizona* was isolated from hens in each group. *P. arizona* was isolated from 49 percent of the vent swabs of unvaccinated hens following challenge as compared to 18 percent of the vent swabs of vaccinated hens.

All eggs laid by the challenged hens were cultured after eight-day incubation. The group of six unvaccinated hens went out of production soon after challenge. There were no isolations of *P. arizona* from the five eggs laid by this group. The six hens that were vaccinated with a broth-type commercial bacterin laid 74 eggs following challenge. *P. arizona* was isolated from one egg laid 19 days after challenge and from one egg laid 20 days after challenge. The six hens that were vaccinated with the oil emulsion bacterin laid 49 eggs following challenge. There were no isolations of *P. arizona* from these eggs.

TRIAL 2

A commercially prepared broth bacterin was used to vaccinate all breeder hens of one ranch and the results compared to experiences of the previous season. These breeder hens were inoculated at the beginning of the laying season, reinoculated four weeks later and given a third inoculation six weeks following the second. Production and fertility for the
vaccinated hens were considered to be good throughout the laying season. Eggs and poults were routinely submitted to the laboratory for culture. These samples remained negative for *P. arizona* during the first fifteen weeks of production. The first isolations of *P. arizona* were obtained from eggs laid five weeks after the last vaccination of the hens.

During the breeding season of the previous year, when unvaccinated hens were maintained on the ranch, *P. arizona* was isolated from eggs and poults early in the laying season and at irregular intervals throughout the season.

**TRIAL 3**

Challenge of day-old poults was used as an indirect method of measuring the immunity developed in hens vaccinated with a bacterin. Progeny of vaccinated and unvaccinated hens were challenged by yolk sac inoculation of *P. arizona*. Each challenged poult received one ml of inoculum containing approximately 12,000 organisms. The first trial involved poults hatched from eggs gathered two weeks after vaccination of their dams. Their dams had received two inoculations of a broth bacterin given at a two-week interval. Mortality in progeny of vaccinated hens was 23 out of 50 poults challenged. Mortality in progeny of unvaccinated hens was 37 out of 53 poults challenged. Twenty poults from both vaccinated and unvaccinated hens were kept as controls. There were no losses in the controls during the 10 day trial period.

The second trial involved poults hatched from eggs gathered four weeks after vaccination of their dams. The mortality in progeny of vaccinated hens was 16 out of 41 poults challenged. The mortality in progeny of unvaccinated hens was 21 out of 28 poults challenged.

The average mortality after challenge was 43 percent in day-old poults from vaccinated hens as compared to 71 percent in day-old poults from unvaccinated hens.

**DISCUSSION**

The trials involving use of a bacterin to immunize turkey breeding stock against *Paracolon arizona* have indicated:

1. There is a reduction in the shedding of *P. arizona* when vaccinated breeders are exposed to the organisms, therefore vaccination may be expected to curtail the spread of *P. arizona* within a flock.
2. Vaccination may also protect the hen from systemic infection, thus preventing transmission of *P. arizona* through the egg.
3. Parental immunity is transmitted to poults of vaccinated hens.

Vaccination of turkeys against *Paracolon arizona* appears to be beneficial, however it is not considered as a substitute for other disease control procedures, but rather as an adjunct to sanitary measures.
PARACOLON ARIZONA CHALLENGE
OF DAY OLD POULTS

Progeny of Vaccinated Hens

Percent
Mortality
Two Weeks
Post Vaccination

Progeny of Non Vaccinated Hens

Days After Challenge

0 1 2 3 4 5 6 7 8 9 10

Total 65%
23/50

Total 70%
37/53

Days After Challenge

Progeny of Vaccinated Hens

Percent
Mortality
Four Weeks
Post Vaccination

Progeny of Non Vaccinated Hens

Days After Challenge

0 1 2 3 4 5 6 7 8 9 10

Total 39%
16/41

Total 75%
21/28

Days After Challenge

Figure 1
INFECTIOUS BOVINE RHINITIS ABORTION

Joseph A. Molello, D.V.M., Ph.D.*

Infectious bovine rhinotracheitis (IBR) was observed first in Colorado feedlot cattle in 1950, but was not reported in the literature until 1955.9 The clinical conditions caused by IBR virus are not limited to the respiratory system as its name implies. The diverse pathogenicity of IBR virus is indicated by its etiologic role in conjunctivitis,1 vulvovaginitis,5 balanoposthitis,13 and encephalomyelitis.3 These clinical entities occur in beef and dairy types of cattle. Jensen et al.,4 1955, reported that pregnant heifers infected with the virus frequently aborted. However, it has been only in the last three or four years that abortion due to the IBR virus has been recognized as a serious and widespread syndrome in cattle.

Recently, Pierson and Vair12 reported on the economic losses caused by IBR infection in a dairy herd. In addition to abortions, there was a marked drop in milk production and calves which were also affected developed a mild respiratory disorder, opacity of the cornea and meningitis. Virtually all manifestations of IBR infection were encountered in the herd. Although all clinical entities induced by IBR infection are of economic importance, the abortion syndrome appears to be increasingly significant as more is learned about the abortifacient capability of the virus. In considering a definitive diagnosis for cases of bovine abortion, not only should brucellosis, leptospirosis, trichomoniasis, listeriosis, nutrition and other factors be considered, but the possibility of IBR should not be overlooked. Although our discussion is limited to a review of diagnostic features in regard to IBR abortion, the approach applies to the investigation of other infectious conditions as well.

Herd history may provide some suggestion of IBR infection when bovine abortions occur. The presence of an upper respiratory condition preceding abortion by as much as the past 202 to 648 days may be an indication of herd exposure to the IBR virus. Respiratory infection with IBR virus is generally accompanied by elevated body temperatures of 103 to 107°F and other clinical signs which may include salivation, cough, rhinitis with a nasal discharge that is serous initially and then becomes mucopurulent, depression, keratoconjunctivitis and decreased milk production. The disease may be disseminated in a susceptible herd either rapidly or slowly and progressively extending over a month or more. Detectable maternal illness may not precede abortion but at least some individuals in the herd usually will have exhibited some signs which suggest the presence of IBR infection.

The vaccination program for the herd should be considered since it has been reported that IBR vaccine can induce abortion.8 It appears that the incidence may be greatest if vaccination occurs during the sixth, seventh, or eighth month of gestation.

*From the Department of Pathology and Toxicology, Pitman-Moore Company, Division of Dow Chemical Company, Indianapolis, Indiana.
Experimentally, inoculation of heifers with a field strain of IBR virus late in the first trimester of pregnancy resulted in abortion within twenty-four days and inoculation of pregnant heifers late in the second trimester was followed by abortion in about twenty days postinoculation. Conceivably, abortion due to IBR virus may possibly occur at any stage of gestation, since the critical effect of the virus is exerted primarily on the fetus. However, most abortions under field conditions seem to occur at about the fourth to seventh month of pregnancy. Consideration should be given to the possible role of the virus as a cause of neonatal death. This may be particularly true if maternal infection occurs late in gestation and insufficient time elapses for the virus to be fatal to the fetus. Neonatal death may not be associated with any respiratory system virus and the role of IBR infection may be over-looked. Additional studies are indicated since some neonatal deaths may be due to intrauterine infection with IBR virus. Kruinengen recently reported the death of a ten day old calf as being due to IBR infection so it seems that IBR infection may be of significance at any time.

IBR abortion may be accompanied by retention of an edematous placenta in which cotyledons usually are blanched and degenerated due to a generalized necrotizing placentitis. Traction, however, may be required to remove the membranes.

Characteristic gross lesions in the aborted fetus have not been reported. Autolysis and subcutaneous serosanguineous fluid and hemoglobin-tinged fluids in the body cavities are often seen but merely indicate intra-uterine death of the fetus at about twenty-four to forty-eight hours prior to abortion. Occasionally, the liver may appear pale or the fetus may be icteric.

Microscopic lesions in fetal tissues, although not definitive, provide strong presumptive evidence of infection with IBR virus. A focal necrotizing hepatitis as well as focal areas of necrosis in the spleen, kidney, thymus, lymph nodes, myocardium and brain have been reported. Foci of coagulative necrosis may be found in other organs. Fetal death is due to the viremia which appears to be responsible for the focal necrosis in the liver as well as in any of the other organs. The placenta frequently shows a generalized necrosis. This appears to be the result of slow fetal death which gradually deprives the placenta of adequate circulation, the resultant hypotension and anoxia being responsible for the generalized necrosis and edema. Intranuclear inclusions found in other forms of the disease have not been reported in fetal tissues.

Presumptive evidence of IBR abortion may be confirmed by serologic studies, virus isolation or animal inoculation.

The standard test for IBR antibody is a serum neutralization procedure in a primary bovine kidney tissue culture system. Demonstrable antibodies may develop as early as ten days after onset of the disease and titers are usually maximum four to six weeks following onset of the infection. High titers may persist for months. Therefore, the time the serum samples are selected in order to demonstrate a rise or decline of serum antibody levels is important if a serologic diagnosis of IBR infection is to
be made. Because subclinical infections of IBR appear quite common, the mere presence of serum antibodies should not be considered sufficient evidence for a diagnosis of IBR infection. The demonstration of a decline in serum antibodies is difficult since antibody levels persist for such long periods. A rise in titer is meaningful and usually can be demonstrated more readily. However, proper timing in selecting paired serum samples is most difficult to achieve because of the subclinical nature of the infection in so many instances. Nevertheless, paired samples two or three weeks apart, from normal appearing, clinically affected and recovered animals should be examined. Thus, serum samples from several members of the herd should be submitted for serologic studies. This approach provides the best opportunity for meaningful serologic information because serum from females that have aborted may have reached a maximum titer when abortion occurs thereby precluding the opportunity to demonstrate a rise in titer. Successful tests are greatly augmented if blood is obtained as aseptically as possible and the serum decanted prior to submitting the samples to the laboratory. This minimizes bacterial contamination and hemolysis which frequently negate the efforts to determine antibody titers in bovine embryo kidney cell (BEKC) systems.

Unequivocal diagnosis can be made on the basis of virus isolation. Placenta and a wide variety of fetal tissues and body fluids are sources of IBR virus in cases of abortion. Tissues should be selected as aseptically as possible and frozen as soon as possible. They then can be packed in dry ice and forwarded to the laboratory. The isolation is accomplished by the inoculation of tissues into a BEKC system. Virus activity is indicated as a cytopathogenic effect (CPE) characterized by disruption of the cell sheet with clumping of rounded, granular cells. This change may occur within twenty-four hours of inoculation and intranuclear inclusion bodies may be found in many of the cells when fixed in Bouin's solution and stained with hematoxylin and eosin. The CPE is neutralized by specific IBR antiserum. Susceptible cattle may also be used for virus isolation. Infection following inoculation is usually characterized by clinical signs similar to the disease under field conditions. IBR virus can be reisolated from nasal washings on about the seventh day but usually no later than ten to fourteen days. Leukopenia also can be demonstrated on about three to six days postinoculation. Serologic tests can be used to demonstrate IBR antibody response. The clinical response, reisolation of the virus and serologic results can be used to confirm the diagnosis of IBR virus infection when susceptible cattle have been inoculated with an isolate.

In summary, the following features may be helpful in recognizing IBR abortion in cattle:

1. A herd history that indicates the presence, either currently or recently, of any or all of the following clinical signs:

   A mild upper respiratory syndrome prior to abortion, contagious keratoconjunctivitis, meningoencephalitis of undetermined origin in calves, pustular vulvovaginitis, balanoposthitis, fetal death about twenty-four to forty-eight hours prior to abortion and retained, markedly edematous placenta with pale cotyledons.
2. Histopathologic lesions consisting of focal necrosis of liver, kidney, spleen as well as in other organs.
3. Serologic evidence of IBR based on an increase in serum antibody titers, indicating recent exposure to IBR virus.
4. Isolation of IBR virus from placentas, fetal tissues or fetal body fluids.

REFERENCES
HYPOMAGNESEMIA—A DIAGNOSTIC ENIGMA

David C. Kradel, D.V.M., M.S.¹

University Park, Pennsylvania

One primary responsibility of a diagnostic laboratory is to determine what is causing illness or death in livestock. In many cases, the "what" is apparent, but the "why" is usually less obvious and is one of the most frustrating problems encountered by those advising livestock owners. Acute hypomagnesemia not only creates a problem in determining what, but is challenging scientists to determine why.

Sudden death in livestock with few or no observed premonitory signs is not uncommon. Such deaths usually go uninvestigated because they account for a very small percentage of total disease mortality and because they usually so surprise the owner that disposal of the carcass is his only immediate impulse. It would be interesting to know just how many "acute" deaths do occur in any one county or state during a year. Such information is probably not available.

The following case reports are typical examples of one condition in which acute death can be the only sign.

Case #1.¹² The owner of a 28-cow commercial Agnus herd had experienced losses of from one to three lactating cows for the past six years. The dead cows were usually over six years old, in average physical condition, and nursing calves four weeks to five months old. Usually, the cows were found dead with no premonitory signs. One cow had been found alive in a semi-comatose condition and lived two days before dying. In November of 1961, the owner found another nine-year-old cow dead, and the cow was presented to the diagnostic laboratory. Gross necropsy, histological, heavy metal toxicological, bacteriological, and animal inoculation examinations were essentially negative. The history and absence of other specific findings suggested the possibility of hypomagnesemia. Blood samples were taken from seven of 21 lactating cows and analyzed for calcium and magnesium (Tables 1 and 2 - 12/8/61). Dietary supplementation with concentrate and magnesium (magnesium sulfate added to ration so that each cow received about 18/gram/magnesium/day) was begun and blood sera again analyzed after one and two months. (Tables 1 and 2 - 1/5/62

¹Dr. Kradel is at the Animal Diagnostic Laboratory, Department of Veterinary Science, Pennsylvania State University, University Park, Pennsylvania. The author gratefully acknowledges the help of the following people: Dr. Robert Marshak for calcium and magnesium determinations in Case 2; Dr. Jack Phillips, Dave Fowler and Bob Scarth, Department of Animal Industry and Nutrition, Pennsylvania State University, for providing the animals and information for Case 2; Dr. J. B. Washko, Department of Agronomy, Pennsylvania State University, for providing soil analyses of the pastures; and, Mrs. Ida Harris and Mrs. Elizabeth Geiger for technical help and manuscript preparation.
Case 1. Calcium Content of Serum Samples from Cattle Prior to and Following Dietary Supplementation Beginning on December 9, 1961

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Case 1. Magnesium Content of Serum Samples from Cattle Prior to and Following Dietary Supplementation Beginning on December 9, 1961

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$^1$Least Significant Difference.

and 2/8/62). Magnesium supplementation was continued in succeeding years, and no further losses occurred from 1961 through 1964.

Case #2. This herd consisted of 86 crossbred beef cows. The cows were kept on a forage-pasture program with no concentrate supplementation. They had been randomly divided into two groups of 43 each when put to pasture in the spring of 1965. The pasture and water source for the two groups were thus different. Between 6/3/65 and 6/24/65, four cows from the one group were found dead. Three had
shown no previous signs of illness. One had "trembled severely" when released from a chute in which she had been checked for pregnancy on the day previous to her death. The dead cows were three to four years old and were nursing five- to eight-month-old calves. Extensive pathological, toxicological (heavy metals, insecticides, fluorides, alkaloids, thallium), microbiological, and animal inoculation studies were negative. Water from the pasture where losses had occurred was given to rabbits and mice for eight weeks with no ill effects. Blood from 10 cows in each herd of 43 was analyzed with the results as shown in Tables 3, 4, and 5. Soil samples from the pastures where

### TABLE III
Case 2. Blood Values from 10 Cows in Herd Where No Losses Occurred

<table>
<thead>
<tr>
<th>Cow</th>
<th>WBC</th>
<th>Hb.</th>
<th>PCV</th>
<th>Ca mg %</th>
<th>P mg %</th>
<th>Mg mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7650</td>
<td>10.7</td>
<td>38.5</td>
<td>8.16</td>
<td>7.00</td>
<td>1.94</td>
</tr>
<tr>
<td>17</td>
<td>9100</td>
<td>10.2</td>
<td>38.0</td>
<td>9.16</td>
<td>5.39</td>
<td>2.06</td>
</tr>
<tr>
<td>37</td>
<td>6150</td>
<td>10.7</td>
<td>39.0</td>
<td>8.72</td>
<td>4.83</td>
<td>2.01</td>
</tr>
<tr>
<td>47</td>
<td>8500</td>
<td>9.4</td>
<td>33.0</td>
<td>8.84</td>
<td>7.27</td>
<td>2.18</td>
</tr>
<tr>
<td>51</td>
<td>8000</td>
<td>11.0</td>
<td>41.0</td>
<td>9.94</td>
<td>5.17</td>
<td>2.66</td>
</tr>
<tr>
<td>64</td>
<td>11000</td>
<td>10.2</td>
<td>33.5</td>
<td>10.96</td>
<td>5.61</td>
<td>2.32</td>
</tr>
<tr>
<td>74</td>
<td>11300</td>
<td>9.9</td>
<td>34.0</td>
<td>9.16</td>
<td>6.27</td>
<td>2.20</td>
</tr>
<tr>
<td>91</td>
<td>11150</td>
<td>11.4</td>
<td>41.5</td>
<td>11.86</td>
<td>5.95</td>
<td>2.33</td>
</tr>
<tr>
<td>95</td>
<td>10400</td>
<td>10.2</td>
<td>31.5</td>
<td>9.44</td>
<td>6.34</td>
<td>2.51</td>
</tr>
<tr>
<td>97</td>
<td>8200</td>
<td>10.7</td>
<td>37.0</td>
<td>10.60</td>
<td>5.83</td>
<td>2.71</td>
</tr>
<tr>
<td>Average</td>
<td>9145</td>
<td>10.4</td>
<td>36.7</td>
<td>9.68</td>
<td>5.97</td>
<td>2.29</td>
</tr>
</tbody>
</table>

### TABLE IV
Case 2. Blood Values from 10 Cows in Herd Where Losses Occurred

<table>
<thead>
<tr>
<th>Cow</th>
<th>WBC</th>
<th>Hb.</th>
<th>PCV</th>
<th>Ca mg %</th>
<th>P mg %</th>
<th>Mg mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6850</td>
<td>9.6</td>
<td>35.5</td>
<td>11.96</td>
<td>5.75</td>
<td>0.86</td>
</tr>
<tr>
<td>54</td>
<td>8900</td>
<td>10.7</td>
<td>37.0</td>
<td>11.14</td>
<td>6.46</td>
<td>1.07</td>
</tr>
<tr>
<td>69</td>
<td>8950</td>
<td>8.5</td>
<td>32.0</td>
<td>10.26</td>
<td>6.98</td>
<td>1.37</td>
</tr>
<tr>
<td>71</td>
<td>8700</td>
<td>10.2</td>
<td>35.0</td>
<td>10.66</td>
<td>8.28</td>
<td>0.87</td>
</tr>
<tr>
<td>104</td>
<td>7700</td>
<td>10.5</td>
<td>37.0</td>
<td>9.78</td>
<td>6.74</td>
<td>1.31</td>
</tr>
<tr>
<td>110</td>
<td>8200</td>
<td>10.2</td>
<td>36.0</td>
<td>10.40</td>
<td>7.52</td>
<td>0.76</td>
</tr>
<tr>
<td>116</td>
<td>9350</td>
<td>10.2</td>
<td>35.0</td>
<td>8.90</td>
<td>7.20</td>
<td>1.14</td>
</tr>
<tr>
<td>132</td>
<td>9150</td>
<td>10.2</td>
<td>36.5</td>
<td>10.80</td>
<td>6.59</td>
<td>1.21</td>
</tr>
<tr>
<td>134A</td>
<td>14900</td>
<td>8.5</td>
<td>32.0</td>
<td>10.66</td>
<td>7.32</td>
<td>1.17</td>
</tr>
<tr>
<td>134B</td>
<td>12150</td>
<td>9.4</td>
<td>32.0</td>
<td>11.00</td>
<td>7.10</td>
<td>1.09</td>
</tr>
<tr>
<td>Average</td>
<td>9485</td>
<td>9.8</td>
<td>34.8</td>
<td>10.56</td>
<td>6.99</td>
<td>1.09</td>
</tr>
</tbody>
</table>
the two groups had pastured were analyzed with the results as shown in Table 6.

### TABLE V
Case 2. Summary of Blood Values and Soil Potassium and Magnesium Levels

<table>
<thead>
<tr>
<th></th>
<th>WBC</th>
<th>Hb, gms</th>
<th>PCV (%)</th>
<th>CA, mg%</th>
<th>P, mg%</th>
<th>K(soil), lb/acre</th>
<th>Mg(soil), lb/acre</th>
<th>Mg(sera), mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd with losses</td>
<td>9485</td>
<td>9.8</td>
<td>34.8</td>
<td>10.56</td>
<td>6.99</td>
<td>258</td>
<td>100</td>
<td>1.09</td>
</tr>
<tr>
<td>Herd with no losses</td>
<td>9145</td>
<td>10.4</td>
<td>36.7</td>
<td>9.68</td>
<td>5.97</td>
<td>266</td>
<td>140+</td>
<td>2.29</td>
</tr>
</tbody>
</table>

Soil mineral determinations are on basis of available elemental mineral/acre for Mg and available K₂O for K.

### TABLE VI
Case 2. Soil Sample Analyses from Pastures

<table>
<thead>
<tr>
<th>Pasture</th>
<th>PH</th>
<th>Phosphorous</th>
<th>Potassium</th>
<th>Organic Matter</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.2</td>
<td>21</td>
<td>210</td>
<td>High</td>
<td>140+</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>23</td>
<td>160</td>
<td>High</td>
<td>140+</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td>95</td>
<td>350</td>
<td>High</td>
<td>140+</td>
</tr>
<tr>
<td>4</td>
<td>6.2</td>
<td>33</td>
<td>450</td>
<td>High</td>
<td>140+</td>
</tr>
<tr>
<td>5</td>
<td>6.2</td>
<td>8</td>
<td>160</td>
<td>High</td>
<td>140+</td>
</tr>
<tr>
<td>8A</td>
<td>5.7</td>
<td>20</td>
<td>280</td>
<td>High</td>
<td>82</td>
</tr>
<tr>
<td>8B</td>
<td>6.3</td>
<td>20</td>
<td>300</td>
<td>High</td>
<td>86</td>
</tr>
<tr>
<td>12</td>
<td>6.5</td>
<td>21</td>
<td>195</td>
<td>High</td>
<td>116</td>
</tr>
</tbody>
</table>

Cows ingroup with no losses had been on pastures 1, 2, 3, 4, 5. Losses occurred in cows rotated on pastures 8A, 8B, and 12. Mg is reported on the basis of available elemental Mg/acre. 140 is the top Mg level checked, so values could be much higher. K is reported on the basis of available K₂O/acre.

**DISCUSSION**

The primary purpose of this paper is to suggest that acute hypomagnesemia may be easily overlooked by the laboratory diagnostician; and that when it is considered, it is difficult to prove.

The cases presented are representative of what the author believes is typical acute hypomagnesemia in Pennsylvania beef herds. The evidence to be presented supports but does not prove this hypothesis.

In Case 1, the average serum magnesium value of 1.35 mg percent with two of seven cows having values below one milligrams percent suggests that subclinical hypomagnesemia was present in some of these
animals (Table 2). "Normal" serum magnesium values range between 1.22 to 3.53 mg percent with an average of 2.80 mg percent. It is well recognized that fluctuations occur and may be influenced by individual physiological differences, season of the year, nutrition, and changing climatic conditions. A marked drop in serum magnesium has been commonly observed in cows grazing the lush pasture of spring, but clinical hypomagnesemia does not necessarily occur. Nonetheless, serum magnesium levels near or below one mg percent as found in several cows in Case 1 and Case 2 (Tables 2 and 4) can be indicative of potential trouble. The factor or factors that precipitate a clinical crisis are largely unknown. Sudden weather changes and exertion have precipitated some cases. It is interesting to note that in Case 1 the cows with the lowest magnesium levels also had the lowest calcium levels (Tables 1 and 2). Simultaneous hypocalcemia-hypomagnesemia has been associated with clinical tetany.

The response of the cows in Case 1 to supplementary magnesium and concentrate is apparent with the average serum magnesium going from 1.35 to 2.04 mg percent (Table 2). Magnesium intake is certainly one factor in maintaining normal blood magnesium levels. In Case 2, the soil magnesium level in the pastures where losses occurred was lower than in the pastures where no losses occurred (Table 6). The cattle in the low magnesium containing pastures had a much lower serum magnesium level (1.09 mg percent average, Table 4) than did the cows in the higher magnesium containing pastures where no losses occurred (2.29 mg percent average, Table 3).

Clinical hypomagnesemia can be prevented with magnesium supplementation. In Case 1, yearly losses ceased after beginning magnesium and grain supplementation. Most beef herds with acute hypomagnesemia cases that we have observed were not receiving grain supplementation; and, there does appear to be a relationship between T.D.N. (total digestible nutrient) intake and the occurrence of hypomagnesemia.

Magnesium intake is not the only factor governing blood magnesium levels. There appears to be rather conclusive evidence that K/Ca+Mg ratio of pasture grass may be an important factor, with high ratios creating potential problems. In Pennsylvania soils, levels above 150 lbs/acre of available K2O are considered high and those above 200 lbs/acre very high. The potassium levels in Case 2 were thus all high or very high (Tables 5 and 6). High levels of nitrate have also been shown to decrease magnesium utilization.

Physiological differences in the ability to absorb, utilize, or mobilize magnesium is also suggested by several observations. Clinical cases usually involve only a very small number of the total herd and more commonly involve mature or older cattle in which mineral mobility may be decreased. Also, some cow families are more susceptible to hypomagnesemia. Three of the four dead cows in Case 2 were sired by one bull, and the sire of the fourth was not known. Siring of the
43 cows in this group represented 10 bulls. The dams of the dead cows also had superior milking records.

Clinical hypomagnesemia is thus the end result of complex interrelationships involving the animal, its environment, and its nutrition through the plant. The plant, in turn, is influenced by the interrelationships of soil nutrients.

In Case 2, the 86 cows had been fed, housed, and otherwise identically maintained until divided when turned to pasture. The fact that one group developed subclinical and probable clinical hypomagnesemia demonstrates the complexity of the above relationships. Nutritional factors (pastures and water?) must have operated to create subclinical hypomagnesemia, but physiological differences (three of four cows known to be sired by the same bull) may have accounted for the clinical crisis.

It is the author's opinion that acute hypomagnesemia may extract a greater loss in livestock than is generally recognized. Clinical, chronic hypomagnesemia is apparently infrequent and seldom recognized. The possibility that it could exist in a herd experiencing occasional acute hypomagnesemic episodes and be responsible for subtle, poorly defined problems has not been intensively studied.

No attempt has been made to cover all the described syndromes associated with hypomagnesemia. The author has superficially reviewed the "what" and the "why" of acute hypomagnesemia. The interested reader is referred to the appended bibliography if he desires more detailed information.

SUMMARY

Acute hypomagnesemia should be suspected when acute deaths occur in mature, lactating, non-supplemented cows and other reasons for death cannot be found. Clinical hypomagnesemia results from complex interrelating factors involving the animal, the plant, the soil, and the environment; but, an actual or conditioned magnesium deficiency is the final result. Magnesium supplementation of the animals or the pasture is a practical solution to the problem.

REFERENCES


THE ROLE AND FUTURE OF DIAGNOSTIC SERVICES

C. D. Van Houweling, D.V.M.,* and E. P. Pope, D.V.M.**

With the establishment of Diagnostic Services for the Animal Health Division at the National Animal Disease Laboratory, Ames, Iowa, there was the expectation that general diagnostic reference assistance for animal disease would be available. When this unit at the National Animal Disease Laboratory was not able to supply all these services, those who had expected them were disappointed. It will be the objective of this presentation to first outline very briefly some of the activities which have been carried on and second, to discuss the policies related to determining what activities shall be undertaken by this laboratory. We will also discuss the limitations that exist at the present time.

The primary role of Diagnostic Services at the National Animal Disease Laboratory has been to provide laboratory services for cooperative state-federal animal disease eradication programs. Such major eradication programs as tuberculosis, brucellosis, hog cholera and the diagnosis of vesicular diseases therefore assume major importance and top priority in the planning of the programs and work of the Animal Health Division personnel. This emphasis is directly related to the budgeting for the activities at the laboratory. First of all, the Division receives funds for specific eradication and control programs such as bovine tuberculosis, brucellosis, hog cholera, screwworm and miscellaneous diseases. The programs of Diagnostic Services are financed from these appropriations. The Division director and his staff must decide within the funds available for any given program, how the funds will be utilized and how much will be invested in laboratory activities. Therefore, the laboratory activities are in competition with the field stations and staff functions for the funds that are available for any given program. This naturally tends to keep the laboratory activities very closely geared to program needs. This program or project orientation actually prevents or discourages laboratory activities that are not directly related to cooperative state-federal eradication program needs. Therefore, it is the natural result that the activities of Diagnostic Services at the National Animal Disease Laboratory are closely related to these programs. The Technical Services laboratory group at Beltsville is more closely related to external parasite programs which rely to a greater extent on the use of chemicals as eradication tools. Technical Services thus assumes those responsibilities in the Division.

The facilities available for laboratory examinations and the holding of

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experimental animals are now completely occupied with activities that
relate directly to the principal Division cooperative programs mentioned
above. In addition to the regular diagnostic functions associated with the
eradication programs, we do engage in developmental activities designed
to improve our present diagnostic techniques and procedures or develop
the utilization of an accepted procedure for a new purpose or a new dis-
ease. We also attempt to demonstrate the effectiveness of new procedures
that may be developed in research and made available to diagnostic lab-
oratories for their use as diagnostic tools. These developmental projects
are also funded from disease eradication programs and therefore are
closely connected with their objectives and goals.

Diagnostic Services has available one-eighth of the laboratory space
at the National Animal Disease Laboratory and approximately one-tenth
of the facilities for holding animals utilized in experimental investigations.
Within this space we have over 50 people engaged in diagnostic and de-
velopmental activities. There is at this time very little room for the ex-
pansion of services because of the limitations on space in which personnel
can work.

State and regional diagnostic laboratories through the Conference of
Veterinary Laboratory Diagnosticians have requested reference assistance
be provided by a national diagnostic laboratory. Those that have been
made known to us are:

1. Technical training and development courses for state diagnostic
   laboratory personnel.
2. Reference assistance for such agents as clostridia, salmonella,
   leptospira, coliform organisms, etc.
3. The development and production of reagents for diagnostic tech-
   niques such as the fluorescent antibody conjugate.
4. Consultation visits to state diagnostic laboratories upon their re-
   quest for such assistance.
5. The development of new or improved laboratory diagnostic tech-
   niques that can be established in state diagnostic laboratories.
6. The authorization of Diagnostic Services through the office of the
   Director of Animal Health Division, Agricultural Research Serv-
   ice, United States Department of Agriculture, to enter into coopera-
   tive agreements with state diagnostic laboratories to carry out
   investigations of livestock and poultry diseases of mutual interest.

We have been able during the past years to meet some of these re-
quests for assistance through the provision of training and the supplying
of diagnostic reagents and other materials.

Training has become one of the major activities of Diagnostic Serv-
ices. The training programs have primarily been supplied to state and
federal veterinarians engaged in the field aspects of control and eradica-
tion programs. We have had several schools to help train these full-time
employees in the differential diagnosis of poultry diseases, hog cholera
and foreign animal diseases. Seminars and conferences have been held for
the epidemiologists working in the tuberculosis and brucellosis programs.
We have been pleased to have the opportunity to present to workers from other laboratories some of the techniques and procedures utilized in our laboratory. These have been provided to pathologists, virologists, technicians, microbiologists both at our laboratory and at regional conferences where techniques and procedures have been demonstrated. We have distributed a limited amount of diagnostic reagents, such as the PK-15 cells and the conjugate necessary for fluorescent antibody hog cholera diagnostic tests. We have served other laboratories as a reference center for Salmonella serotyping, and in the field of mycobacteriology and brucellosis as a reference center and culture repository. A major activity of the laboratory has always been the production of the diagnostic reagents required for brucellosis eradication. These are produced and distributed throughout the world and in quantities necessary to meet all this country's program needs.

We have also developed a competency in the area of clinical pathology as it relates to toxicologic conditions. In many instances, infectious conditions affecting animals are indistinguishable from the symptoms or lesions caused by toxic compounds. A diagnostic capability must be available to make accurate diagnoses and distinguish these conditions from infectious diseases, especially those suspected of having a foreign origin. In this area we are prepared and stand ready to function as a reference center for other diagnostic laboratories.

We understand why the veterinary diagnostic laboratories of this country desire to have the same service made available to them as is provided to the public health laboratories by the Communicable Disease Center, Atlanta, Georgia. Unfortunately, the limitations of space at the National Animal Disease Laboratory will not make it possible to materially expand the services that we have been able to supply to state diagnostic laboratories. We are cognizant of the need for the services which can be provided by a national laboratory. The provision of such activities will require the availability of additional facilities for this purpose. We recognize that as our agriculture has changed, we, the state and federal laboratories, have not kept abreast with the demands for our services. If we do not accelerate our efforts to provide these services, we are going to get further behind.

We see the need of highly competent state diagnostic laboratories backed up by a highly competent federal reference laboratory service. Whether this materializes depends on how well we can present these needs and their significance to the state and federal departments of agriculture, and the livestock and poultry industry.

They must be convinced this need has a high priority among the numerous requests they receive.

Without increased facilities and funds, we will not be able to expand our services. In some areas we will have to restrict our activities because of increasing work loads.

The role and future of a national reference laboratory would appear to be dependent upon the degree of future implementation of the resolutions that have been adopted by this conference.
70th ANNUAL MEETING
October 14–19, 1966
HILTON HOTEL
Buffalo, New York

71st ANNUAL MEETING
October 16–20, 1967
WESTWARD-HO
Phoenix, Arizona

72nd ANNUAL MEETING
October 6–10, 1968
JUNG HOTEL
New Orleans, Louisiana