PROCEEDINGS

EIGHTY-NINTH ANNUAL MEETING

of the

UNITED STATES ANIMAL HEALTH ASSOCIATION

P. O. Box 28176
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THE MARC PLAZA HOTEL
MILWAUKEE, WISCONSIN
October 27–November 1, 1985
This 1985 Proceedings of the U.S.A.H.A. is dedicated to the memory of Dr. George C. Cilley, Jr., Concord, New Hampshire. Dr. Cilley was a member of the Executive Committee representing his state of New Hampshire as State Veterinarian.

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# Record of Previous Meetings

<table>
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<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tr>
<td>15. Dec. 5-6, 1911</td>
<td>Chicago, Ill.</td>
<td>*Dr. John F. Devine, Goshen, N.Y.</td>
<td>*Mr. Wm. P. Smith, Monticello, Ill.</td>
</tr>
<tr>
<td>22. Dec. 2-4, 1918</td>
<td>Chicago, Ill.</td>
<td>*Dr. M. Jacob, Knoxville, Tenn.</td>
<td>*Mr. Wm. P. Smith, Monticello, Ill.</td>
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<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
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<tr>
<td>43. Dec. 6-8, 1939</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wyo.</td>
<td>Dr. Mark Welsh, College Park, Md.</td>
</tr>
<tr>
<td>44. Dec. 4-6, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. A. Crossman, Boston, Mass.</td>
<td>Dr. Mark Welsh, College Park, Md.</td>
</tr>
<tr>
<td>45. Dec. 3-5, 1941</td>
<td>Chicago, Ill.</td>
<td>*Dr. I. S. McAdory, Auburn, Ala.</td>
<td>Dr. Mark Welsh, College Park, Md.</td>
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<tr>
<td>46. Dec. 2-4, 1942</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>58. Nov. 10-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. H. F. Wilkins, Helena, Mont.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>60. Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. G. H. Good, Cheyenne, Wyo.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, Fla.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
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<tr>
<td>Oct. 15-18, 1963</td>
<td>Albuquerque, N.M.</td>
<td>*Dr. T. J. Grennan, Jr., Providence, R.I.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>*Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>Nov. 7-12, 1976</td>
<td>Miami Beach, Fla.</td>
<td>H. E. Goldstein, Columbus, Ohio</td>
<td>*Dr. W. L. Bendix, Richmond, Va.</td>
</tr>
<tr>
<td>Nov. 2-7, 1980</td>
<td>Louisville, Ky.</td>
<td>B. W. Hawkins, Ontario, Or.</td>
<td>Dr. J. C. Shook, Hyattsville, Md.</td>
</tr>
<tr>
<td>Nov. 7-12, 1982</td>
<td>Nashville, Tenn.</td>
<td>G. B. Rea, Salem, Or.</td>
<td>Dr. J. C. Shook, Hyattsville, Md.</td>
</tr>
<tr>
<td>Oct. 21-26, 1984</td>
<td>Ft. Worth, Tex.</td>
<td>J. O. Pearce, Jr., Okeechobee, Fla.</td>
<td>Dr. J. C. Shook, Annapolis, Md.</td>
</tr>
</tbody>
</table>

+ This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION AND MEMORIAL SERVICE

A. J. Roth, D.V.M.
Richmond, Virginia

Heavenly Father we thank thee for the honor of being together at the 89th Annual Meeting of the United States Animal Health Association and the 28th Annual Conference of the American Association of Veterinary Laboratory Diagnosticians.

We are so respectful that you have provided us with the wisdom and perseverance to achieve strides towards continued control and eradication of animal and poultry diseases in these United States.

Hopefully, you will guide and assist us in making of decisions and developing policies that will be beneficial and rewarding to the livestock and poultry industry which we serve.

We are so appreciative of the guidance you have provided us in the past. May it continue for this meeting and other Animal Health meetings in the future.

Amen

Memorial Service

Mr. President, Members of the Association, Ladies & Gentlemen:

Each year the United States Animal Health Association takes time to pay tribute to those members who have passed away since your last meeting. They are:

Dr. William L. Downey—Camp Hill, Pennsylvania, September 1985
Dr. W. W. Kirkham, Panama City, Florida, July 24, 1985

Would everyone please rise and bow your head for a moment of silent prayer.

Amen
It is a pleasure and an honor to welcome the 89th Annual Meeting of the U.S. Animal Health Association and the 28th Annual Conference of the American Association of Veterinary Laboratory Diagnosticians to Wisconsin and to Milwaukee.

You are now in America’s Dairyland: every automobile license plate will remind you of that fact.

Dairying contributes more than 60% of the 5 billion dollars in cash receipts from sale of agricultural products in Wisconsin. The almost 2 million milk cows on about 42 thousand farms place Wisconsin first among the 50 states in production of milk, butter, and cheeses of the common varieties: American, munster, brick, Italian, and blue. Wisconsin concedes first place in Swiss cheese production to Ohio—, without snide comment.

In 1984, almost 7,500 live dairy cattle and calves were shipped out of Wisconsin to international markets. In each of recent years, between 100 to 150 thousand dairy cattle and calves were shipped out of Wisconsin to other states.

It follows that Wisconsin is first among the states in production of hay and in corn for silage.

Not incidentally, it bears mention that this state is first in mink pelts as well as in green peas, in snap and lima beans, beets for processing, and cabbage for sauerkraut.

Clearly, Wisconsin is number one in production of bull semen for use in AI of dairy and beef cattle. In 1984, 49% of the 16.1 million units of frozen bull semen sold domestically or internationally was processed in or by organizations located in Wisconsin. This means more than 8 million “contacts” with heifers or cows located not only in each of the fifty states but, also, “contacts” with heifers or cows in about seventy-five other countries on four other continents.

I can testify that fulfilling this obvious responsibility in respect to the implied disease transmission potential of bull semen has required the utmost in communication, understanding, cooperation, and mutual respect between industry and State and Federal veterinary authorities. To a remarkably high level, these requisites have always been present in Wisconsin.

Wisconsin has had and continues its serious commitments to animal agriculture and dedication to animal health. Last month, Secretary Ausmann of the Wisconsin Department of Agriculture, Trade & Consumer Protection and Acting President Lyall of the University of Wisconsin System jointly announced creation of a “Wisconsin Dairy Task Force 1995” with three primary objectives:
1. to encourage research, education and legislative programs to improve profitability and efficiency of milk and meat production;

2. to improve dairy processing efficiency and enhance product quality;

3. to increase dairy marketing efficiency from the farm to the consumer.

The University of Wisconsin's excellent new College of Veterinary Medicine will produce its first graduates in 1988.

Madison is the location of the National Wildlife Health Laboratory, a unique facility dedicated to the study of diseases of waterfowl.

Wisconsin's Animal Health Division has been and continues to be among the leaders in initiating, developing, and successfully implementing animal health programs.

—Traditionally, Wisconsin has been a leader in bovine tuberculosis and brucellosis programs.

—In recent years, a Johne's vaccine has been evaluated, a major Johne's survey conducted, and a program for herd classification and for documented Johne's free status has been implemented.

—A pseudorabies pilot project and a pseudorabies eradication program are underway.

—A Toxic Response Team is on stand by to provide immediate action and efficient investigation when animal poisoning is suspected.

—Plans are underway for establishment of an equine quarantine station.

—A system by which herds may become certified as bovine leukosis free is well along in its development.

Madison is the site of Wisconsin's Central Animal Health Laboratory, an organization that has not only been able to maintain its "can do", progressive, leadership policies, but, also, consistently provides excellence in service.

It is this laboratory's commitment to providing the numerous special tests essential to satisfy the frequent, unique demands of importing countries — the customers of the USA for animals and animal products — that makes possible not only Wisconsin's international trade in animals and animal products but, also, the similar trade of other states.

My first participation in the meetings of this United States Animal Health Association (then the United States Livestock Sanitary Association) began 38 years ago. It has been my good fortune and to my benefit to have been able to attend most subsequent meetings since 1947.

The fact that I know these meetings of the USAHA and AAVLD consistently provide an annual forum for presentation of the newest in animal disease research, the latest in animal disease problems, and some visibility of formulating policies has impelled me to attend whenever possible. I
am grateful to USAHA and to the AAVLD. I know that all I have said is or will become equally true for most of you.

The growth in depth and complexity and the accomplishments of the science in veterinary medicine are exemplified in this year's program. If only those responsible for the political economics of agriculture were achieving as well!

Finding direction in these times of economic distress in animal agriculture is a major challenge now additive to the continuing challenge of controlling infectious diseases within our livestock populations. We are also challenged to satisfy the requirements of many customer nations desirous of exchanging their cash — which the USA needs — for our animals and animal products.

If the USA is serious — I know Wisconsin is — in an intent to compete successfully in export of animals and of animal products, it follows that both the efforts of the animal industries and the efforts of the State and Federal agencies, i.e., diagnostic laboratory, research, and administrative units, must be no less timely, efficient and effective than those of the countries with which the USA is in active competition for sales.

Our traditional concerns with tuberculosis, and brucellosis, with which we sometimes seem to have become quite comfortable, continue to be of importance. However, realistically, these named diseases are not often the ones creating the crises in our export trade today.

We seem to have no substantial, constructive programs moving toward coping with or overcoming the costly consequences of having to use certain immunological tests, with finality, that are notoriously at variance with actual presence of their respective etiological agents. For cattle, this is especially true with bluetongue, enzootic hemorrhagic disease, and Johne's. How are we to cope with the subtle infections such as leukosis, IBR/IPV, and BVD in cattle, and atrophic rhinitis in swine, that are significantly precluding export sales? For these, new, individual, and effective solutions must be sought. Wisconsin is losing major cattle sales to Saudi Arabia because that customer demands prior foot and mouth vaccination. There must be a way —.

During the course of these meetings, it is my hope that there is no disagreement from any quarter upon any of the following observations I believe relevant:

1. that uniform applications of meaningful test procedures are an absolute necessity for successful exportation of animals and animal products;
2. that successful exporting requires being able to efficiently and effectively satisfy needs and wants of customers, repeatedly, and that repeat business rises and falls with the integrity of exporters;
3. that failure to prosecute willful violators of regulations is a distinct disservice to exporters who conscientiously try to comply with regulations.

If possible, take a few hours to enjoy a bit of Milwaukee. The food is great. The lakefront is remarkable. The Milwaukee Public Museum, just two blocks north and east of this hotel, has many outstanding, life-size American Indian and wildlife exhibits.

And, in closing, I'd especially like to thank Governor Earl, Lieutenant Governor Flynn, and Secretary of Agriculture Ausman whose nonavailabilities have provided to me this opportunity to extend our warm welcomes to Wisconsin and to Milwaukee.
RESPONSE TO WELCOME
Michael J. McDonald, D.V.M.

Thank you Dr. Bartlett for the most gracious invitation to America's Dairyland. On behalf of the United States Animal Health Association and the American Association of Veterinary Laboratory Diagnosticians, I wish to express our sincere appreciation for your hospitality.

As a newcomer in the regulatory field of veterinary medicine, I feel uniquely privileged to be a part of this esteemed group. Never before has the challenge been so great as it is today to aid the American farmer in producing healthy animals and products in the face of mounting and increasingly difficult economic pressures. Our challenge is clear... we must control animal disease effectively with a minimum of impediments to farmers and producers. Our accomplishments in the past are many and will be in the future a direct result of all our efforts to work together to supply America and a large part of the world with the very best animal products.

On behalf of America's Bluegrass State, I wish to invite you to Louisville for our 1986 meeting. Louisville has seen many exciting changes in recent times. Kentucky's most exciting new attraction is the Kentucky Derby Museum. Its spectacular 360 degree multi-media production is one of the largest in the world and captures the adventure and pageantry of the Kentucky Derby. Images of the Derby can be seen, felt, and heard. Louisville will be poised and ready to show our organization why she is one of America's top ten cities.

Thank you and see you in River City.
INTRODUCTION OF FEATURED SPEAKER

David U. Walker, D.V.M.

Our speaker this evening is a long time friend and valued contributor to many aspects of the United States Animal Health Association and is currently serving as Vice Chairman of the Foreign Animal Disease Committee.

Born in Greensboro, NC, and raised in Florida and upstate New York, he is a 1954 graduate of the New York State College of Veterinary Medicine at Cornell University.

After two years in practice he returned to his alma mater obtaining a masters degree in 1959. Then from 1959-1961 was a research associate at the University of Pennsylvania in Clinical Pathology.

The next 13 busy years were devoted to a position in research at Plum Island Animal Disease Center as Assistant to the Director. For 9 of those years he was also responsible for Biological Safety Operations.

He then accepted an assignment with the ARS, National Program Staff at Beltsville with a major responsibility for foreign animal diseases. 1979 found Dr. Hyde as a member of an 8 person team visiting the Peoples Republic of China in an exchange sponsored by the National Academy of Sciences and PRC. This was the first Animal Agriculture Team visiting PRC since establishment of normalization.

In 1980 he became chief staff veterinarian for International Operations in Emergency Programs with APHIS. 1981 found him as national program leader for Animal Health Research.

Retirement followed on July 2, 1984. This slowed him very little because of an immediate 8-month assignment with FAO in the Republic of Korea.

Ladies and gentlemen, I give you Jack Hyde.
UNITED STATES LIVESTOCK AND WORLD MARKETS

John L. Hyde, D.V.M., M.S.*
College Park, Maryland

This evening, I will address the role of U.S. Livestock in world trade, especially in the less-developed countries, the LDC's.

Historically, the United States has been the world leader at some time in almost everything traded internationally. Our strengths — our competitiveness, scientific expertise, and business knowhow, and our talent for innovation — have always given us an advantage.

But today, the United States is losing in many areas of international trade, especially in agricultural trade. The question is, why? The reasons are complex. They go beyond purely economic factors. They involve such things as domestic and foreign policy, perceptions about quality, cultural habits, and dietary taboos. There are a lot of other reasons but they all add up to lost markets that must be replaced or regained.

Despite these complicated problems, opportunities abound for the U.S. livestock industry in international trade. For example, our germplasm is in great demand. And with more research and development in the new areas of biotechnology, trade in germplasm will certainly increase in both imports and exports. This rapidly increasing germplasm trade is raising the pressure to find out as quickly as possible, which diseases are transmissible through semen and embryos. Since many diseases are potentially transmissible, the studies will be necessarily time consuming. We have, then a tension between what is economically desirable — rapid growth in germplasm trade — and what is safe — a reasonable certainty that the germplasm we export — and especially the germplasm we import will not carry diseases that might adversely affect animal agriculture. In the long run, the extra time needed to study the transmission potential of the various diseases with proper care will give us that extra edge of quality so important to today's trade. It should be added, however, that we have imported live animals and semen from countries where diseases exist or might exist that are exotic to the United States. So, in the short term this is an area that needs to be considered especially now that procedures are perfected and commonly used for obtaining so called germ-free or gnotobiotic animals. This might be more appropriate for some species than others.

Recently, I read a report published by the Economic Research Service on "High Value Agricultural Products."(1) Here is an area where we can make more inroads. Instead of sending raw products to other countries that then make them into a final product in high demand, why don't we make these

*Formerly National Program Leader, Foreign Animal Diseases, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD.
products here? Actually, some are made here, but apparently the potential for income from high value agricultural products is not fully appreciated. Also, a high value product industry creates new job opportunities. The benefits of exporting more high value agricultural products from the United States, according to the report, could be significant.

We don’t even know what other countries want in the way of final or semiprocessed animal products. Many Americans think if we like it, so will others. We are guilty of a cultural conceit. Sometimes we are right; but often we are wrong. So, we need to strengthen our knowledge through education and strong consumer marketing studies, along with better marketing practices. This should increase our position in world trade of raw, semiprocessed, and high-value products.

Most Americans who travel and mix with people in foreign countries realize that American products are generally regarded as the best one can buy. There are exceptions, of course. For the most part, these are related to culture, taste, religious beliefs, transportation, and problems with storage and/or preservation. These factors must be addressed in expanding our world trade.

If it is also important to remember that as we help underdeveloped and developing countries through international programs, their standards of living and per capita income will, in most cases, increase. This opens new markets for agricultural products and we must be in a position to provide as many of these products as possible and thus enhance our world trade position and balance of payments. However, we must export quality products whether it is animals or animal products. When we fail, our competitors are there waiting to take over these markets. Research here and overseas can improve standards of living and open new markets for our products.

In 1981, the Office of Technology Assessment (OTA), at the request of the Congress, issued a report entitled “An Assessment of the United States Food and Agricultural Research System.” This report caught the attention of certain Congressional committees, research agencies within the United States Department of Agriculture (USDA), and the Agency for International Development (AID). The Agricultural Research Service, for one, reacted positively by developing a 6-year plan that identified high priority research programs and their implementation. This plan has recently been revised to reflect a changing agricultural and world economy, and it will be updated as needs and priorities change.

One part of the OTA report addresses the development of U.S. international research activities. This primarily related to AID but also included the USDA. As stated in the report, “through early 1981, AID was not organized or staffed to be effective in carrying out its responsibilities. Technical leadership was lacking in the decision-making positions. With 50 percent of the total budget in food and agricultural activities, technical personnel trained in these areas account for 5 percent of the total personnel. Few, if any, were in decision-making positions.” Since 1981, AID
has attempted to respond to the OTA report. Recently, it issued a document entitled “A Strategic Plan for AID.”

From the mid 1950’s through the 1960’s, ARS scientists stationed in Kenya (East Africa) made significant contributions in research on diseases exotic to the United States. These contributions were of extreme value to the United States as well as to other countries. For example, the first diagnostic test for African swine fever was developed along with other research findings that provided information important in understanding the epidemiology, pathogenesis, and the development of better diagnostic tests for African swine fever. An effective vaccine for contagious bovine pleuropneumonia resulted primarily from the activities of ARS scientists working in East Africa. A highly significant research breakthrough on East Coast Fever was made through the establishment of a Lymphoblast tissue culture system infected with a causative organism of East Coast Fever. This had potential then as a vaccine and more recently is being examined as a source of material for a noninfectious, genetically engineered vaccine for that disease. There were many other important research accomplishments made by ARS scientists working in East Africa.

The U.S. Department of Agriculture, especially ARS, could do much more in international research on animal health and production. Today there is only one ARS scientist working on animal diseases in countries where diseases exotic to the United States exist. This, based on past experience, is unfortunate since we cannot duplicate inside a laboratory like Plum Island that research which requires field conditions. On the other hand, the Animal and Plant Health Inspections Service, a regulatory and service agency, continues to do well in their international activities as the record shows. Their programs have also expanded in recent years. There are invaluable opportunities for animal health people, whether in research or service and regulatory activities, to gain first-hand knowledge under field conditions, not only of animal diseases and production practices, but also the countries and their people.

Many universities are in a position to play a significant role in international livestock activities. This is being done to some extent under the Small Ruminant Collaborative Research Support Program. However, to be really effective, universities need a foreign service corps made up of people qualified to work overseas who have faculty status, tenure, and could periodically return to teach and pursue work related to the individual’s interest and talent. It is unfortunate when a university functions as a contracting body and deducts significant overhead in order to find and backstop individuals outside the university for an assignment overseas. This often results in sending persons who are not necessarily the best qualified and then trying to backstop them by short-term visits to see how all is going.

This whole subject is one that needs to be seriously reviewed by the AID, the USDA, universities, and the State Department. It is from the State Department that other departments and agencies must obtain permission
to place people in countries overseas. The State Department's record as far as approving positions overseas for work in agriculture is, in my opinion, not good.

Finally, private companies wishing to pursue trade in many countries have difficulties. Much of this is due to restrictive U.S. laws and regulations which impede their activities. Certain business practices accepted in other countries are considered unethical or illegal by the United States. Our competitors are not so restricted and take full advantage.

There are some other points in the international arena that I believe we should address because some can affect World trade. We are the major source of funding for many international institutions engaged in agricultural research work. Some of these have a fair representation of American scientists on their staffs. In my opinion, most do not. The United States contributes 25 percent of all monies going into United Nations programs and, in most cases, more than that for the International Agriculture Research Centers - such as The International Rice Institute, Wheat and Maize Institute, Potato Institute, International Livestock Center for Africa, and the International Laboratory for Research on Animal Diseases. There are 10 of these centers plus three related programs that receive large financial support from the United States.

The question is "why aren't more Americans involved in international organizations?" For example, let's consider the United Nations (UN), and within the UN, the Food and Agriculture Organization (FAO) and United Nations Development Program (UNDP). One reason more Americans do not work within these organizations is we do not have young people with enough international experience to qualify. Secondly, many do not understand the employment procedures including salaries and benefits, and, thirdly, we do not understand or even appear interested in what these organizations are supposed to do.

On the subject of international experience, there are several European countries and Japan participating in the "Associate Expert Program of the United Nations." This program, which is for 2–3 years' duration, is primarily for young people who are interested in working within the United Nations system, especially the FAO and UNDP. When I was in Korea, I had the pleasure of working with one such young man from The Netherlands. The home country pays the individual’s salary and expenses. In other words, it is like a scholarship. Some individuals in our country have tried to stimulate United States interest in this program, but so far without success. With very little investment compared to future gain, the United States could fund scholarships to obtain technical and administrative experience for individuals interested in working within the framework of the United Nations. We might then begin to have more positive input into international programs, at the technical and administrative levels. This could also serve as a pool of international talent from which governmental agencies, universities, and private business could find knowledgeable individuals to guide them in their international ac-
tivities including world trade. The very fact that most of our competitors are so active in this program indicates that it must pay off. As an example, the following three charts provide information about the UN. Associate Expert Program within the FAO.

**FAO ASSOCIATE EXPERTS**

<table>
<thead>
<tr>
<th>Country</th>
<th>No. Experts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>44</td>
</tr>
<tr>
<td>Denmark</td>
<td>39</td>
</tr>
<tr>
<td>France</td>
<td>11</td>
</tr>
<tr>
<td>Germany</td>
<td>10</td>
</tr>
<tr>
<td>Italy</td>
<td>32</td>
</tr>
<tr>
<td>Japan</td>
<td>10</td>
</tr>
<tr>
<td>Netherlands</td>
<td>93</td>
</tr>
<tr>
<td>Sweden</td>
<td>21</td>
</tr>
<tr>
<td>Switzerland</td>
<td>22</td>
</tr>
<tr>
<td>United States</td>
<td>0</td>
</tr>
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**Areas of FAO Associate**

<table>
<thead>
<tr>
<th>Expert Participation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>143</td>
</tr>
<tr>
<td>Asia/Pacific</td>
<td>55</td>
</tr>
<tr>
<td>Latin America</td>
<td>52</td>
</tr>
<tr>
<td>Europe/Near East</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
</tr>
</tbody>
</table>

**Fields of Specialization**

<table>
<thead>
<tr>
<th>in FAO and UNDP</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Hydrology</td>
<td>Veterinary Entomology</td>
</tr>
<tr>
<td>Irrigation</td>
<td>Fisheries Technology</td>
</tr>
<tr>
<td>Soil Survey</td>
<td>Forestry</td>
</tr>
<tr>
<td>Botany</td>
<td>Agricultural Planning</td>
</tr>
<tr>
<td>Home Economics</td>
<td>Agricultural Marketing</td>
</tr>
<tr>
<td>Technology of Food Products</td>
<td>Activities related to the</td>
</tr>
<tr>
<td>Land Settlement</td>
<td>World Food Program</td>
</tr>
<tr>
<td>Animal Production and Health</td>
<td></td>
</tr>
</tbody>
</table>

I have a few slides to refresh your memory about world land use and selected livestock production before discussing where we may be more active in World trade.

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*Personal Communication: Dr. William Moulton, East Dover, Vermont.*
WORLD LAND USE AND SELECTED LIVESTOCK PRODUCTION**

TOTAL LAND (Billions Ha)**

<table>
<thead>
<tr>
<th></th>
<th>Developed Countries</th>
<th>Developing Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arable</td>
<td>1.4 47%</td>
<td>53%</td>
</tr>
<tr>
<td>Crops</td>
<td>0.1 23%</td>
<td>77%</td>
</tr>
<tr>
<td>Pasture</td>
<td>3.2 40%</td>
<td>60%</td>
</tr>
<tr>
<td>Wood</td>
<td>4.1 45%</td>
<td>55%</td>
</tr>
<tr>
<td>Other</td>
<td>4.3 39%</td>
<td>61%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>13.1 42%</td>
<td>58%</td>
</tr>
</tbody>
</table>

TOTAL LIVESTOCK (Billions)**

<table>
<thead>
<tr>
<th></th>
<th>Developed Countries</th>
<th>Developing Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>1.2 35%</td>
<td>65%</td>
</tr>
<tr>
<td>Sheep</td>
<td>1.1 47%</td>
<td>53%</td>
</tr>
<tr>
<td>Pigs</td>
<td>0.8 44%</td>
<td>56%</td>
</tr>
<tr>
<td>Goats</td>
<td>0.5 5%</td>
<td>95%</td>
</tr>
<tr>
<td>Total</td>
<td>3.6 37%</td>
<td>63%</td>
</tr>
<tr>
<td>TOTAL MEAT PRODUCTION (million MT)</td>
<td>145.0 62%</td>
<td>38%</td>
</tr>
</tbody>
</table>

MILK PRODUCTION**

<table>
<thead>
<tr>
<th></th>
<th>Developed Countries</th>
<th>Developing Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Milk Cows (millions)</td>
<td>226 50%</td>
<td>50%</td>
</tr>
<tr>
<td>Total Milk Production (million MT)</td>
<td>438 83%</td>
<td>17%</td>
</tr>
<tr>
<td>Total Goats (millions)</td>
<td>473 5%</td>
<td>95%</td>
</tr>
<tr>
<td>Goat Milk (millions MT)</td>
<td>8 27%</td>
<td>73%</td>
</tr>
</tbody>
</table>

**Personal Communication: Dr. Charles Haines, U.S. AID.
DEVELOPING AND UNDERDEVELOPED COUNTRIES

Africa

Ruminants found in the northern part of the Western Hemisphere, with some exceptions, do not do well in most tropical countries in Africa. This is especially true of high-producing dairy breeds. They are highly susceptible to tropical diseases, and grain and forages are usually not available to support the needs of these animals. In addition, husbandry and management practices are usually not sufficient to supply the care and attention these animals require. Through the use of selective crossing of large ruminants and future genetic manipulations, this may, in part, be overcome, especially for the beef breeds.

Goats and sheep are important and highly regarded animals in most countries in Africa. This is an area offering good potential in the United States for exports and also for importation of disease-resistant goats and sheep germplasm for crossing with desirable breeds here. If this cross-breeding cannot be done in the United States, it may be possible overseas. Future technology developed through research may open the doors for importation of certain kinds of germplasm free of infectious diseases.

The role of the chicken is well established and could become even more so with increased marketing research, transfer of technology, and through genetic improvements. For example, studies could be done to develop birds that are more resistant to adverse environmental factors such as heat.

There are other avian species which should be considered for hot, tropical climates and where grain may be in short supply. For example, the Kaki Campbell Duck is unique in that it does very well under adverse environmental conditions, lays about 200 eggs per year, forages for itself, and is resistant to several serious diseases that affect chickens (4). Also, there are indigenous wild birds such as small partridges that do very well in parts of Africa. The success of introducing new species of animals or new management concepts is dependent upon how well people will accept them. If, however, demonstrations can show something is helpful, it may become accepted.

In Africa, the pig is an excellent source of red meat where religious beliefs do not prohibit the eating of pork. Being omnivorous, it readily adapts to a variety of feed sources. In fact, swine and poultry production in many developing countries are located primarily near major population areas where “left-overs” from man serve as the main source of food for these animals. Here again, genetic selection and cross breeding to produce pigs which more readily adapt to the heat, humidity, and other adverse conditions in parts of Africa are important in capturing our share of exports.

Latin America

In Latin America, the situation is or can be very different from tropical Africa. There have been successful enterprises undertaken with North
American dairy breeds, and management practices are changing to adapt to the requirements of dairy farming. There should be more expansion in this area and, hopefully, we will be there to take advantage of it. One problem in many countries of the world including Latin America is transportation and preservation of perishable products. Refrigeration is not widespread, especially outside of large cities.

The Danes and Dutch have essentially captured the world market as far as the sale of fluid milk. They market and sell sterile milk produced by ultrahigh-temperature (UHT) processing. Such milk can be found in most countries, especially where refrigeration is a problem. Some may say dried milk is the answer, but try that on young people or older ones use to the taste of fluid milk. Apparently this was a major problem for the United States Navy until it started purchasing UHT processed milk. Milk is a commodity that more and more people will desire, especially if it is available throughout their lifetime.

Our beef germplasm is sought by many Latin American countries, and they have germplasm we want and, to some extent, have acquired. The economic benefits to those countries and to the United States could be significant.

Poultry production is doing much better in Latin America but can be improved even more, especially with the increase of living standards in certain countries. As in Africa, goats and sheep are valuable animals in some countries in the Caribbean and in Latin America. This is an area that requires more marketing studies and education to realize their importance and justify increased exports of these animals. This is especially true if we can show that one high-quality animal can be more valuable than two less desirable ones. If properly managed, goats and sheep are an excellent source of food and fiber, and can do well in many areas where cattle are difficult to raise.

**OPEC Countries**

The United States used to be the major exporter of agricultural products to the OPEC countries. This has changed, and in large part it is our fault. We have let other countries take over. They are supplying high-value products, well packaged and attractive. I have heard stories of frozen U.S. chickens that were obviously crushed during freezing or accidental thawing and were not very appealing when displayed in stores in these countries. This is a quality-control problem.

Also, in addition to price competition by other developed countries, we are not marketing, selling, demonstrating, or exhibiting our products. It would appear that good old-fashioned advertising, sales promotion, and good business practices are needed. However, here again, we run into the problem of our own ethical standards preventing us from conducting business according to the cultural traditions and expectations of our prospective trading partners.
Asia

The potential in Asia is enormous; it holds about half of the Earth's population. As Asian countries become more developed, we should realize important markets, provided we determine their needs. What do they like or what might they like? Culture is important, but will probably change faster in many countries of Asia than in other parts of the world as individual incomes rise. This is evident by the increasing number of U.S. "fast-food" restaurants appearing in certain large cities. Having recently returned from the Republic of Korea, I have no doubt that Koreans want to try new things and acquire more animal products to vary their source of dietary protein.

Aquaculture

Aquaculture, or fish farming, is becoming more and more recognized as an excellent, source of meat protein which requires significantly less economic inputs compared with other animal species. With the exception of a few States, we have been slow to realize the potential of fish farming. The United States produces less than 15 percent of the fish we consume. There are people who see aquaculture significantly expanding in the United States. However, more people in decision making positions must begin to appreciate this if funds for research are to increase.

In some Latin American countries, aquaculture could be a major source of protein as well as increased income from exports. Some countries rely on the sea, but there are other countries with large fresh-water supplies that could grow fish commercially. Some are doing so now, but infrastructures are needed to make this practical and economical. The South of Chile is noted for its excellent trout, but whether the true commercial value of such a highly valued product is fully appreciated is not known.

There is an opportunity for U.S. expertise to participate in these ventures by helping these countries to realize a cash flow beneficial to them, and thus open markets to the United States.

In the People's Republic of China, in 1979, I saw fish farming at its best. Part of the reason may be the desire for meat protein and the value and ease of raising fish as a source. However, they also know how to cook fish such as carp, and it tastes as good as any fish I have eaten. The same thing has occurred in Japan, Southeast Asia, and to some degree, in Korea. However, Korea and Japan rely mostly on the sea as a source of protein.

The new dietary guidelines recently issued for Americans are once again encouraging us to eat lean meats such as fish and chicken. A message often repeated may well sink in and last.

It was a pleasure to hear a representative of the National Cattleman's Association on the MacNeil-Lehrer News Hour indicate that the cattle industry realizes it must change in order to meet the demands of the public. This may take some time, but it is a positive approach.

Of course, what is published as U.S. dietary guidelines is not necessarily
accepted by other countries. Most underdeveloped and developing countries limit their intake of red meat simply because they cannot afford it. I once heard it said that "people who have been on a diet primarily of grain with a little fish or chicken now and then as a source of meat protein will change their way of eating when their income permits. When that happens, they want red meat." This is the sort of thing the red meat industry has to do indepth market studies on to determine how to best capture much of this enormous market that is rapidly evolving in many developing and some underdeveloped countries.

Future Implications from Research

Biotechnology will certainly play a big role in the future. You have all heard the great possibilities, perhaps with some degree of apprehension. For example producing cotton fibers in fermentation tanks. If that can happen to plants, what about animals? Well, remember oleomargarine. The dairy industry fought it, but lost the battle. This is always the chance with new technology. If it comes, you either get out, change, or stay with what you have in the hope that some people will prefer the "real thing" and pay more to get it. Changes will certainly continue, and hopefully for the betterment of mankind. We must adapt to inevitable changes or lose out. So far, we have led the world in agricultural production because our producers have changed with the times while others have waited.

Future implications from research were well stated in a paper presented before the Brookings Institution by Terry Kinney, Administrator of ARS(6). In summarizing his paper, Dr. Kinney said that "Focusing our research resources primarily on increasing agricultural production no longer seems as urgent as it did a few years ago. There is currently a world food production surplus. Finding new uses for U.S. agricultural commodities — and helping our farmers make a satisfactory profit — seem to be far more realistic priorities." Dr. Kinney gave examples such as recent research which found a way to change beef tallow, a surplus item in the United States, into cocoa butter utilizing a lipase enzyme. This is not yet ready for commercial use, but it illustrates changes that occur to put our agriculture into a more competitive position by converting surplus commodities into products that are in demand in the marketplace.

Our wealth of technology should be looked upon as one of our most valuable high quality export items. By entering into joint business ventures with developing countries who need our technology, this could be economically very advantageous. It seems we have been giving but not receiving.

As stated earlier, it is important to remember that as underdeveloped and developing countries improve their standards of living and per capita income, there will be new markets for agricultural products including livestock and livestock products. We must be ready to provide as many of these products as possible and enhance our world trade position. However, we must export quality. When we fail, our competitors are there to take over.
So, what is the role of U.S. livestock in world trade? It is big and will increase. However, those who will succeed are like those who made it in the past — those who are willing to adjust to change, undertake strong consumer marketing research studies, sell high-quality animals or animal products, and who believe in hard work and competition. It is essential that we send individuals overseas who are internationally oriented and well trained. Finally, in a free enterprise environment, where free trade is not a one way street, we will reaffirm that if it is labeled, stamped, branded, or certified as originating in the United States, it's the best you can buy.

Thank You.

REFERENCES


4. Personal Communication: Dr. Charles W. Beard, USDA, ARS, SEPRL, P.O. Box 5657, Athens, Georgia.

5. Personal Communications: Dr. Dyarl D. King, USDA, ARS, Beltsville, Maryland.

MESSAGE BY THE INCOMING PRESIDENT OF AAVLD

H. S. Gosser, D.V.M.
Tifton, Georgia

President Walker, President Crandell, Distinguished Guests, Ladies and Gentlemen, I am pleased to have this opportunity to visit with you this evening. I want to briefly discuss some thoughts I have pertaining to the direction and goals of the American Association of Veterinary Laboratory Diagnosticians. What is the American Association of Veterinary Laboratory Diagnosticians? What are its aims? What are its goals? The AAVLD is a conglomerate—a mixture of people, many of whom have specialized in defined disciplines of veterinary medical science. Though from different specialities, these people have one common goal—and that is the detection and diagnosis of diseases of animals. Diagnosticians need to use every available resource, every available piece of information, every possible advantage when attempting to solve disease problems, and many times even these are not enough.

Diagnostic medicine is important to many people in veterinary medicine and to people who either depend upon livestock as a means of livelihood or have pets as companion animals. The AAVLD needs to provide leadership and opportunities for diagnosticians to strengthen themselves so that the optimum service is provided.

One of the aims or goals of the AAVLD is to find ways to improve communications among its members. The AAVLD annual meeting has been and will continue to be a wonderful mechanism for diagnosticians to come together for an interchange of scientific knowledge and to address common problems. During its 28 years of existence, this association has been blessed with leadership by people who have dedicated most if not their entire careers to diagnostic medicine. They have understood the need for a service based on state-of-the-art techniques and an active communication system. Communication is important not only between laboratories, but also between laboratories and state and federal governmental agencies, veterinary practitioners, and the animal industry.

One area which the association must address is to provide a mechanism for the dissemination of information to its constituency. While the annual proceedings of the scientific meetings are a valuable reference in our offices and laboratories, we need a publication which is produced more frequently. A refereed journal published quarterly would provide such a mechanism and would give visibility to the association. It will be a faster means for dissemination of scientific material, such as new and improved diagnostic procedures, case reports and other types of information pertaining to diagnostic medicine.

Another way to improve communication is to have a more active committee structure within the association. This will involve more members and the published committee reports would make information available to more people. Stated annual committee meetings are important and will be
encouraged. Some of the committees have completed their work and perhaps need to be dissolved. Also, there is repetition of a few AAVLD and USAHA committees. I call on the USAHA to join with me to consider the possibility of appointing joint committees where needed so that information is not rehashed within a period of a few days in two separate committees.

Regional workshops, particularly in the area of laboratory techniques, sponsored by the AAVLD would greatly benefit many laboratories and their personnel. A few successful workshops have been held, such as the mycoplasmal diseases of swine workshop at Purdue in 1981 and the mycoplasmal diseases of cattle workshop in San Angelo, Texas in 1982. Most laboratories simply do not have the funds to send laboratory workers great distances to attend workshops. Sessions held at various locations around the country would allow many laboratory workers, especially technicians, the opportunity to share in the new technology available.

Commercial exhibits at annual meetings are a necessity and must be put into place for the 1987 meeting. With the technological advancements and new instrumentation on the market, it is imperative that the diagnosticians have opportunities to see and compare these laboratory instruments. Space would not be a problem once the number of exhibitors is known; only the arrangements would have to be worked out with the hotel.

Another area to be addressed is the laboratory accreditation program. The AAVLD provides the only veterinary laboratory accreditation program in the country and is being called upon more frequently to visit and approve various types of laboratories, such as those limited to poultry, or to wildlife, or to small animal medicine. This provides a challenge that we must address, and we must revise our accreditation guidelines. The AAVLD started the accreditation program and has been instrumental in improving the quality of veterinary diagnostic medicine.

Since its inception, the AAVLD has met many challenges and we must continue to provide an active service for our members. To do this, we must not become static, but continually strive for improvement and provide the best, most up-to-date diagnostic services available. Throughout its history, the AAVLD has been a dynamic organization and has provided the leadership to upgrade veterinary diagnostic programs. President Crandell has continued this leadership and last year at this meeting appointed a Long Range Planning Committee. This committee has been extremely active and has made some rather far reaching recommendations and this committee will be asked to continue its work.

I would like to take this opportunity to remind everyone of the Fourth International Symposium of Veterinary Laboratory Diagnosticians to be held June 2–6, 1986, in Amsterdam, The Netherlands. I hope as many of you as possible will make plans to attend.

We have many challenges before us, and I look forward to working in the coming year with the officers and membership of both the AAVLD and the USAHA to address these challenges. Thank you.
REMARKS BY THE PRESIDENT OF AAVLD

R. A. Crandell,
College Station, TX

Dr. Walker, distinguished guests, ladies and gentlemen,

I would like to thank our host state for the hospitality and fine weather we have been blessed with here in Milwaukee.

This has been a busy year with Board meetings in Florida, Las Vegas and now in Milwaukee. Our committees have been very busy and I wish to express my sincere appreciation to all chairmen and members.

One tends to measure accomplishments by reflecting, but that reflection or vision is sometimes blurred with the things you didn't get done.

However, I was encouraged tonight to hear Dr. Gosser identify some of those challenges which lie ahead. Some were the same as I mentioned a year ago and I believe if people begin to talk about them, they eventually get done. That cleared my vision somewhat. Sometimes the recognition and acceptance of challenges (problems) is the hardest part in solving them.

Our Long-Range Planning Committee has identified and recommended some organizational changes and services within the AAVLD; they believe these changes will strengthen our Association's structure and provide more to the membership. I hope the present enthusiasm continues in this critical review of our organizational structure and activities. However, I urge that we go forward with a steady pace but act with wisdom only after careful examination.

In addition to our regular activities, we sponsored a SNOVET workshop on Saturday with good attendance. Ten states and Canada were represented.

On Sunday, our seminar on the use of computers in diagnostic laboratories attracted approximately 100 participants.

We published our first membership directory with 600 U.S. members and 101 foreign members listed.

Our new Pathology group had a very successful workshop.

Our goal is to provide our membership with the latest developments in diagnostic medicine. Therefore, we must keep abreast with the advances in technology. I commend Dr. Walker’s appointment of the Ad Hoc Committee on Biotechnology which meets tomorrow.

I am happy that AAVLD and USAHA have set in motion a new approach to review our common needs. It is my belief that both parties are committed to continue constructive discussions until a satisfactory agreement is negotiated. It is a must.

I thank Dr. Walker for his cooperation this year, and I wish Dr. Kruse a successful year as president of USAHA in 1986.
I am confident Dr. Gosser will lead AAVLD forward. He is dedicated and a hard worker and I pledge my full support.

Thank you and good night.
REPORT OF THE SECRETARY-TREASURER

J. C. Shook, V.M.D.

Mr. President, distinguished guests, members and friends of the USAHA and AAVLD.

On behalf of the secretary's office we welcome you to Milwaukee. After a cool weekend, Ella has arranged to have beautiful weather the remainder of the week. Our office has been very busy, but with two great gals like Ella and Linda, we have managed to conduct the business and duties of the organization with a minimum of problems.

The organization continues to grow. There are now nearly 1300 paid up members. As in all organizations, with growth comes more work and responsibilities and more expenses for the office of the secretary. Under the able leadership of Dr. Walker, our business practices have been thoroughly reviewed and changes made where indicated to increase efficiency and improve our service. This has included the procurement of a computer for handling our records and accounting. There are many applications for a computer for example: keeping our committee memberships updated and readily available. Please be reminded computers do not think for themselves. We need your changes of address promptly so mail does not go astray.

We want to publicly express our appreciation to Norm and Jay Powers for again very ably handling the registration desk.

The organization is financially solvent. A copy of the cash flow account will be provided to members of the executive committee. Any members who wish a copy can make their request to the Richmond office.

Accept our sincere appreciation for your participation in pre-registration. Approximately 500 have preregistered and there were never any long line waiting at the registration desk.

Be reminded that papers are due in final form at the time of presentation. If we are to get the proceedings out promptly we must have all papers within the week.

Resolutions must be on the proper forms which are available at the workroom. When finished, six copies should be given to J. O. Pearce, Chairman of the resolutions committee or turned in at the registration desk.

Enjoy Milwaukee and have a successful meeting.
UNITED STATES ANIMAL HEALTH ASSOCIATION  
P. O. BOX 28176  
SUITE 205, 6924 LAKESIDE AVENUE  
RICHMOND, VIRGINIA 23228-0176  

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR  
PERIOD JANUARY 1, 1985 through DECEMBER 31, 1985  

CASH BALANCE — DECEMBER 31, 1984:  
Cash on Hand — December 31, 1984 $ 4,300.28  
Bank of Virginia  
Richmond, Virginia  
Checking Account (635.72)  
Savings Certificate 10,000.00  
Savings Account 42,722.54  

$ 56,387.10  

RECEIPTS:  
Individual Dues — 1985 $ 8,721.10  
Individual Dues — 1986 17,375.00 26,096.10  
Official Dues — 1985 12,600.00  
Official Dues — 1986 200.00 12,800.00  
Proceedings 9,129.62  
Reprints 3,818.98  
Foreign Animal Books 32,948.98  
Avian Influenza Books 642.08  
Registrations 25,455.00  
Pre-Registrations 40,843.00 66,298.00  
Ladies Registrations 1,170.00  
Tours 6,759.80  
Photographs 108.00  
Membership Mailing Lists 452.40  
Wednesday Dinner 649.00  
Nat’l Assembly Lunch 21.54  
Rosters 132.14  
Junior Membership 3.00  
Interest 4,561.39  

$165,591.03  

TOTAL BEGINNING BALANCE AND RECEIPTS $221,978.13
DISBURSEMENTS:

Annual Meeting $16,822.61
Printing 32,143.17
Office Supplies (includes computer) 10,256.01
Office Furniture 300.00
Salaries 26,500.13
Office Secretary 10,764.50
Social Security Tax 2,621.33
Federal Unemployment Tax 546.62
Virginia Unemployment Insurance 236.77
Communications 7,043.49
Public Relations Expense 426.29
Retirement — Blanton and Ragland 800.00
Travel:
  Dr. David U. Walker 2,622.62
  Mr. J. O. Pearce, Jr. 902.29
  Dr. J. C. Shook 581.25
  Ella R. Blanton 911.99
  Linda B. Ragland 256.00
Rent — Office Space 4,940.00
State-Federal Meeting 394.38
Shipping Books 1,545.17
Surety Bond 32.00
Safe Deposit Box Rent 17.00
Post Office Box Rent 45.00
Flowers 23.40
Auditing Books 600.00
Insurance 846.86
American Association of Veterinary
  Livestock Diagnosticians 10,625.00
Henrico County Tax 147.17
Bank Service Charges 141.35
Preregistration — A.V.M.A. (Dr. Shook) 85.00

$133,177.40

CASH BALANCE — DECEMBER 31, 1985:

Bank of Virginia
Richmond, Virginia
  Checking Account $3,393.59
  Money Market Savings 52,704.76
  Savings Account 2,702.38
  Certificate of Deposits 30,000.00

$88,800.73
ADDRESS OF THE PRESIDENT-ELECT

N. W. Kruse, D.V.M.
Lincoln, Nebraska

Members of the United States Animal Health Association, members of the American Association of Veterinary Laboratory Diagnosticians, distinguished guests, ladies and gentlemen.

This is certainly the most significant honor and responsibility bestowed upon me in my entire career. I thank the membership of this association for the opportunity to serve in this way. I want to thank my colleagues, both within the north central region and outside for their confidence and reassurance. I also want to thank my wife, Ruth, who has been very cooperative and most tolerant in the past 40 years of practice and my regulatory medicine career.

In reviewing the accomplishments of this organization since its inception in 1897, and especially during the past 15 years which I have observed first-hand, one cannot help but be impressed with the progress which has been made and be grateful to the fine leaders who have been, for a great part, responsible for the accomplishments of the organization.

It is with a great deal of humility that I accept this challenge and responsibility. When I read the roster of past presidents, I realize the awesome challenge of following in the shoes of such industrious leaders. I will try hard to fill them, but I need the assistance and cooperation of all members of this association.

As president-elect, my greatest concerns are for the future. With rapid changes taking place in our agricultural economy, and new emphasis on environmental control and improvement, with new discoveries in technology of disease diagnosis, control and eradication, and the threat of foreign animal diseases being introduced into our country, we need to assert ourselves and let our presence be known. Positive steps also must be taken to eradicate diseases and to carefully evaluate our disease programs for the future.

I feel it high time that we eradicate brucellosis. As Mr. Bert Hawkins has stated, "veterinary services goal is to become brucellosis-free by 1990." We cannot accomplish this goal unless state and federal regulatory personnel and industry all pull together. We must change our attitudes and think positively.

The quarterly newsletter has been a most important vehicle to carry on communication with our membership. However, it is important that lines of communication be wide open both ways, not only from the secretary’s office to members, but also members should keep the officers informed of impending problems in order that appropriate, timely action can be undertaken. We intend to put the newsletter to better use by asking the officers and committee chairmen to use it as a means of disseminating timely information.
Committee chairmen, you do play a very important role in this organization. I would ask you to please keep the ball rolling for the entire year, rather than center on the annual meeting week. I would request that you communicate often with our secretary and the president, and we will reciprocate.

Persons wishing to serve on a committee should contact the chairman of that committee. The chairman, in turn, will confer with the president, who will make the final decision on the appointments. The main office will send out all committee appointments.

The authors of papers will be allowed 15 minutes for speaking and 10 minutes will be allowed for committee reports.

As president-elect of this association, I will ask each and every member of this association to sign up one new member in 1986. Let's see if we can reach this goal.

As an officer of this association, I commend the United States Department of Agriculture for its efforts. As the state veterinarian of Nebraska, I have always been one to give acclaim to the U.S. Department of Agriculture when deserving and have always been critical when necessary. This will not change.

In conclusion, may I thank you for your confidence. I will do my best to provide leadership, and above all I will need your help.
One of the most pleasurable tasks assigned to the president-elect of this organization is that of making the presentation of the association mementoes to the out-going president. At this time, I am extremely proud to present to president Dr. Dave Walker these mementoes as tokens of appreciation for his leadership and direction of this association during the past year.
REMARKS OF THE PRESIDENT

David U. Walker, D.V.M.
Montpelier, Vermont

Distinguished Guests, Ladies and Gentlemen

Lateness of the hour and hardness of these seats lends pointed meaning to brevity. Accordingly, my remarks this evening will be brief.

At this time it is my pleasure to thank a few people in a special way for their very considerable contributions over the past year. First of all I thank our very personable executive secretary, Mrs. Ella Blanton. Ella has again this year performed in outstanding fashion to keep everyone appraised of activities within USAHA. She attends to every small detail to make the annual meeting a splendid affair. Mrs. Linda Ragland our office secretary has worked with diligence and devotion to assure that our records remain up-to-date. Additionally this year she has thrown herself into learning the complexities of computer science.

I stated when taking this important office at USAHA’s annual meeting at Fort Worth that I accepted the honor given me with considerable humility and boundless pride. Having nearly served my tenure as president I now prepare to step down and can honestly admit satisfaction with my challenge. Cooperation which has been afforded me during this time has strengthened my humility. I have not yet been turned down on a single request for help. For that I truly appreciate the membership of this great organization. Recognition of the prestige in which USAHA is held has expanded my pride in our system. USAHA is truly blessed with limitless expertise and a committee system which draws on the maximum of that ability.

Accomplishments over the past year reflect solid progress and forward thinking. I will mention a few which seem most noteworthy. Your board of directors have adopted a system designed to better recognize the contribution of committee chairmen. Our organizational records are at this time nearly completely computerized. A new computer coupled with substantial effort on the part of office staff was required to bring about this accomplishment.

USAHA is now in sound financial condition thanks in large part to the frugality of innovation and diligence of several past presidents. Our insurance program has been substantially upgraded in several areas which include better coverage for office records, the office per se, workmen’s compensation and organizational liability coverage.

During the past year the board capitalized on a sudden opportunity and added one room, an additional storage area to the Richmond office. Ella was highly instrumental in this effort. She poked, she prodded, she per-
sisted and, finally she persevered. This room will be utilized primarily to house our various publications.

An initiative has been started toward the possible writing of a history of USAHA.

Finally an ad hoc committee has been appointed to make recommendations on how best for USAHA to address the rapidly developing field of biotechnology.

I want to thank the board of directors for their diligence and plain hard work in administering the functions of this great organization during my year as president.

Also, I want, most especially to thank the committee chairpersons for their considerable contribution. Many, if not most, of these people have addressed their jobs on a twelve month basis. They are the strength of USAHA. Cooperative input on their part along with input from committee membership is recognized worldwide and programs developed under this system are held in international esteem.

I wish each of you an enlightening and profitable meeting.

Thank you.
Mr. B. W. Hawkins, Administrator, APHIS, VS, USDA, presents Animal and Plant Health Inspection Service's Animal Health Award to Mr. J. O. Pearce, Jr., Rancher and former President of USAHA.

Dr. J. W. Glosser, Associate Administrator, APHIS, VS, USDA, presents for Mr. B. W. Hawkins, Administrator, the Animal and Plant Health Inspection Service's Animal Health Award to Dr. J. G. Flint, former State Veterinarian of Minnesota.
REPORT OF THE COMMITTEE ON NOMINATIONS

President ........................................ N. W. Kruse
Lincoln, Nebraska

President-Elect ................................. John Hudelson
Denver, Colorado

First Vice President ......................... John Cobb
Atlanta, Georgia

Second Vice President ...................... Phil Bradshaw
Griggsville, Illinois

Third Vice President ......................... Max Van Buskirk, Jr.
Harrisburg, Pennsylvania

Treasurer ....................................... J. C. Shook
Annapolis, Maryland

Regional Delegates
Northeast .................................. Everett Bryant
Storrs, Connecticut
Victor LaBranche
Boston, Massachusetts

North Central ............................. Don Gingerich
Parnell, Iowa
Bill Gallagher
Highmore, South Dakota

South ...................................... Joe Finley, Jr.
Encinal, Texas
William Baisley
Dalton, Georgia

West ......................................... Olin H. Timm
Dixon, California
R. H. McCapes
Davis, California
RESOLUTIONS
United States Animal Health Association
Passed November 1, 1985
Milwaukee, Wisconsin

Resolution No. 1
Source: Transmissible Disease of Poultry
Subject Matter: VVND

Resolution
BE IT RESOLVED, that the USAHA at their annual meeting in October at Milwaukee, Wisconsin, recommends that the USDA, APHIS, pursue a more flexible protective program under the Emergency Program that will reduce the budgetary cost involving total depopulation of all pet birds involved in every outbreak, and that the flexibility be determined on a risk ratio factor for each specific outbreak with total consideration to protecting the commercial poultry industry.

Resolution No. 2
Source: Animal Welfare Committee
Subject Matter: Terrorist Activity — Animal Research

Resolution
BE IT RESOLVED that USAHA seek and support legislation making it a Federal crime to break into research laboratories for the purpose of liberating animals, damaging or destroying property, or otherwise conducting terrorist activities which cause the disruption of experimental procedures with animal subjects.
Resolution No. 3
Source: Parasitic Disease and Parasiticides Committee & Epizootic Attack Committee
Subject Matter: Heartwater

Resolution
BE IT RESOLVED, that USAHA urges the U.S. Department of Agriculture and the U.S. Agency for International Development to support funding for research into improved methods for the diagnosis and control of heartwater disease and the control and eradication of its tick vectors.

BE IT FURTHER RESOLVED, that USAHA strongly urges the U.S. Department of Agriculture and the U.S. Agency for International Development to support and fund, respectively, a program for the eradication of the tick Amblyomma Variegatum (and thereby heartwater disease) from the Caribbean region.

Resolution No. 4
Source: Infectious Diseases of Horses
Subject Matter: Equine Salmonellosis

Resolution
BE IT RESOLVED that the USDA, industry organizations, research and clinical groups cooperate in formulating procedures of prevention, control, and quantification of equine salmonellosis, and initiate basic and applied studies of the antibiotic resistance patterns so as to limit further losses and produce a necessary body of scientific knowledge.

Resolution No. 5
Source: Infectious Diseases of Horses
Subject Matter: Vesicular Stomatitis

Resolution
BE IT RESOLVED that USDA be strongly urged to intensify epidemiological studies and research supporting such studies, especially to identify the reservoirs and modes of transmission, but research should not be limited to these facets of this disease.
Resolution No. 6
Source: Infectious Diseases of Horses Committee
Subject Matter: Importation of Horses

Resolution

BE IT RESOLVED the Committee urges USDA and other appropriate agencies to give the highest priority to resolving this situation and correcting import procedures to prevent future problems.

Resolution No. 7
Source: Zoological Animal Committee
Subject Matter: Malignant Catarrhal Fever, Non-Domestic Ruminants

Resolution

BE IT RESOLVED that USDA, APHIS, VS be urged to adopt a policy pertinent to the control of alcelaphine malignant catarrhal fever as follows:

That, all wildebeest (*Connochaetes* spp.) shall be tested for antibodies to malignant catarrhal fever (MCF) virus by the serum-virus neutralization test. This test shall be applicable to incoming and outgoing animals, and

That, wildebeest seronegative for antibodies of MCF shall be eligible for shipment anywhere, provided they have not had exposure to animals with MCF for 60 days prior to shipment, and their test samples are collected no more than 30 days before shipment, and they meet all the requirements of the state of destination, and

That, wildebeest seropositive for antibodies to MCF virus intended for shipment to zoos, domestic or foreign, will not be shipped unless the consignee and the Chief Animal Health Officer of the state or country of destination are informed of MCF seropositive status of the animal, and written approval for shipment has been obtained, and

That, all other categories of ruminants are eligible for shipment without serologic test for antibodies to MCF virus, as only wildebeest are recognized carriers/shedders of transmissible alcelaphine MCF virus, and

That, the serum-virus neutralization test shall be considered positive at $\geq 1:4$ dilution.
Resolution No. 8
Source: Zoological Animals — Import-Export
Subject Matter: U.S. Origin Animals

Resolution

BE IT RESOLVED, that the United States Animal Health Association urges that in addition to the current policy on the movement of offspring of PPEQ animals that the U.S. Department of Agriculture (USDA), Veterinary Services Area Veterinarians in Charge issue permits for the movement into normal commerce of U.S. animals susceptible to FMD and/or rinderpest if it has been determined that the animals have been maintained separate and apart from restricted postentry quarantined animals, have not had direct or indirect contact with restricted animals and upon physical examination are observed to be free of clinical signs of disease.

Resolution No. 9
Source: Zoological Animal Committee Epizootic & Wildlife Committees
Subject Matter: Model Regulations to Control Livestock and Poultry Diseases in Zoological Animals

Resolution

BE IT RESOLVED that the United States Animal Health Association urges the Animal and Plant Health Inspection Service, USDA, to develop a Model Regulation for states to use in the control of livestock and poultry disease in zoological animals.

Resolution No. 10
Source: Committee on Sheep and Goats
Subject Matter: Approval for Drugs For Use in Small Ruminants

Resolution

BE IT RESOLVED that drugs approved for use in goats also be approved for use in sheep.
Resolution No. 11
Source: Committee on Sheep and Goats
Subject Matter: Ovine Footrot

Resolution
BE IT RESOLVED that the USAHA assign to footrot a very high priority among diseases of sheep and support funding of footrot research and vaccine development at the highest possible level for at least 3 to 5 years.

Resolution No. 12
Source: Transmissible Diseases of Swine Committee
Source: Caliciviruses

Resolution
BE IT RESOLVED that the Committee on Transmissible Diseases of Swine strongly endorse continuing the ongoing cooperative calicivirus research programs at the current or increased funding levels for the next three to five years and that additional funds be set aside to initiate a federal in-house calicivirus research program.

Resolution No. 13
Source: Committee on Sheep and Goats
Subject Matter: Scrapie Eradication Program

Resolution
BE IT RESOLVED that the USAHA encourage APHIS to conduct an indepth review of the program to determine the cost/benefit ratio and effectiveness of the program toward eradication of scrapie.

Resolution No. 14
Source: Bluetongue and Bovine Leukosis Committee
Subject Matter: Development and Testing of Bluetongue Vaccines

Resolution
BE IT RESOLVED that the Anthropod-Borne Animal Disease Research Laboratory and its ongoing cooperative research affiliates continue to pursue the development and testing of safe, effective Bluetongue vaccines for the livestock industry of the United States.
Resolution No. 15
Source: Bluetongue and Bovine Leukosis Committee
Subject Matter: Establish Sentinel Surveillance and Economic Impact Statement for Bluetongue 2

Resolution
BE IT RESOLVED that the U.S. Department of Agriculture immediately implement multiple sentinel surveillance programs which would monitor the spread and develop an economic impact assessment of this spreading exotic agent.

Resolution No. 16
Source: Committee on Sheep and Goats & Epizootic Attack Committee
Subject Matter: Nematodirus battus

Resolution
BE IT RESOLVED that USDA be encouraged to establish a surveillance program to determine the incidence, prevalence and distribution of Nematodirus battus

Resolution No. 17
Source: Committee on Sheep and Goats & Epizootic Attack
Subject Matter: Nematodirus battus

Resolution
BE IT RESOLVED that USAHA encourage USDA to develop diagnostic procedures for identification of this parasite.
Resolution No. 18
Source: Foreign Animal Disease Committee
Subject Matter: U.S. Representation in International Organizations

Resolution
BE IT RESOLVED, that the Secretary of the United States Department of Agriculture, the Administrator of the Agency for International Development, and if necessary, the Secretary of Department of State select a small, qualified group to meet in a convenient location to review and make recommendations for consideration by the appropriate Federal Department(s) and Agencies to improve this situation. One area that should be considered is the “United Nations Associate Expert Program.” The United States has participants in this program which is intended to train primarily young people in the technical and administrative areas for future positions as they arise within the various organizations of the United Nations, especially within the Food and Agriculture Organization (FAO) and United Nations Development Program (UNDP).

Resolution No. 19
Source: Committee on Sheep and Goats, Foreign Animal Diseases and Epizootic Attack Committees
Subject Matter: Nematodirus battus

Resolution
BE IT RESOLVED that USAHA encourage USDA to implement anthelmintic trials toward development and approval of effective drugs to control this parasite, and studies to determine the distribution and economic significance of the parasite in the U.S.

Resolution No. 20
Source: Leptospirosis
Subject Matter: Increase In Research Funding

Resolution
BE IT RESOLVED that the United States Animal Health Association urge the Agriculture Research Service and Animal and Plant Health Inspection Service, USDA, to increase funding for both intramural and extramural research in the development of methods for the diagnosis, isolation, propagation and the incidence of leptospires in domestic animals, the typing of leptospira isolates and improvement of leptospiral vaccines.
Resolution No. 21
Source: Epizootic Attack Committee
Subject Matter: Identifying Cattle Moving from Mexico into the U.S.

Resolution
BE IT RESOLVED, that non-registered cattle moving from Mexico into the U.S. must be branded on the left jaw with a 3" letter M or other appropriate symbol. For registered cattle U.S. officials will recognize the same form of permanent identification recognized by Breed Associations providing that the Breed Association is recognized by USDA, APHIS.

Resolution No. 22
Source: Tuberculosis Committee/Import-Export Committee
Subject Matter: Imported Mexican Cattle

Resolution
BE IT RESOLVED, that at the point of entry from Mexico into the United States that all cattle be quarantined and Tuberculosis tested by U.S. officials.

BE IT FURTHER RESOLVED, that changes be made in the CFR to provide for testing these cattle moving in interstate commerce every six months and recommendations be incorporated into the UM&R to provide for testing those which do not move.

Resolution No. 23
Source: Tuberculosis Committee Meeting
Subject Matter: Handling of Tuberculosis Infected Bison

Resolution
BE IT RESOLVED, that the USAHA goes on record as strongly recommending that all bison raised under agricultural conditions shall be handled under the same tuberculosis testing, indemnity and depopulation conditions as cattle except that prior to January 1, 1987, state classification will not be affected by M. bovis infection in bison herds.
Resolution No. 24
Source: Tuberculosis & Johne's Disease
Subject Matter: Standardization of Para TB

Resolution
BE IT RESOLVED, that procedures and funding should be established
to standardize diagnostic tests, antigens, and other reagents for use in the
conduct of tests for diagnosis of paratuberculosis and to approve laborato-
ries to conduct tests for the diagnosis of paratuberculosis.

Resolution No. 25
Source: Tuberculosis/Paratuberculosis Committee
Subject Matter: State Indemnity Provisions for Animals Destroyed Be-
cause of Tuberculosis

Resolution
BE IT RESOLVED that it is recommended that all states review their
regulations for possible revision of indemnity provisions for bovine tuber-
culosis reactor and exposed cattle and bison to insure reasonable participa-
tion.

Resolution No. 26
Source: Tuberculosis & Johne's Disease
Subject Matter: Staffing within Cattle Diseases

Resolution
BE IT RESOLVED, that the USDA should create a staff position to deal
specifically with Johne’s disease to work on regulatory guidelines and
public education.
Resolution No. 27
Source: Tuberculosis Committee
Subject Matter: Tuberculosis Eradication Program

Resolution
The State Veterinarians and the Area Veterinarian in Charge in the Central Region met on September 26, 1985, recommend that the Bovine Tuberculosis Committee of the United States Animal Health Association urge the United States Department of Agriculture to do the following:

BE IT RESOLVED that USDA:
1. Accelerate its efforts in traceback workups of all infected herds.
2. Accelerate Bovine Tuberculosis training efforts nationwide of all State-Federal regulatory veterinarians, and
3. Examine the need for tuberculosis testing of dairy cattle moving in interstate commerce out of the Southwestern States.

Resolution No. 28
Source: Professional Oversight Committee
Subject Matter: Accreditation of Attending Veterinarians

Resolution
BE IT RESOLVED that the USAHA requests that the U.S. Department of Agriculture review and assess the advantages of requiring that veterinarians designated by the licensees and registrants to function under the authorities of the Animal Welfare Act be accredited by the Department. The accreditation may or may not be a part of the current veterinary accreditation program but should include a certification that the veterinarian is sufficiently trained and knowledgeable to assure compliance with the requirements of the Act.
Resolution No. 29
Source: Professional Oversight
Subject Matter: The Management of Slaughter and Livestock Exposed to Rabies.

Resolution

BE IT RESOLVED that the United States Animal Health Association urge the USDA, FSIS, MPI to reevaluate its current policy on the handling and slaughter of livestock exposed to rabies.

FURTHER, BE IT RESOLVED that the USDA, FSIS, MPI develop a policy more in agreement with current scientific knowledge of rabies and those policies of other governmental agencies.

Resolution No. 30
Source: Professional Oversight Committee
Subject Matter: Cattle Fever Tick Treatment on Mexican Cattle Imports

Resolution

BE IT RESOLVED, that treatment procedures at the port of entry be reviewed to determine their effectiveness. This review should include consideration of the appropriateness of the parasiticide utilized and its recommended treatment concentration.

Resolution No. 31
Source: Anaplasmosis Committee
Subject Matter: Attenuated Live Anaplasmosis Vaccine

Resolution

BE IT RESOLVED that the United States Animal Health Association urge USDA to support efforts to develop a live attenuated anaplasmosis vaccine for use in the United States and other countries to protect cattle against anaplasmosis.
Resolution No. 32
Source: Epizootic Attack Committee
Subject Matter: Presence of Resistant Boophilus microplus Ticks in Republic of Mexico

Resolution

BE IT RESOLVED that the USAHA urges Veterinary Services of APHIS to make every effort to make sure that their treatment materials and methods are effective in controlling the insecticide resistant strains of cattle fever ticks; and

That VS/APHIS and ARS/USDA increase cooperative efforts to monitor the presence and spectrum of resistance of cattle fever ticks in Mexico; and

That ARS intensify and increase research at the Cattle Fever Tick Research Laboratory in order to:

(1) Define the chemical, biochemical, genetic mechanisms of resistance in resistant strains,

(2) test new insecticide treatment techniques and other materials, methods, and technologies to find those that are effective against insecticide-resistant strains, and

(3) provide to the Cattle Fever Tick Program information on those materials, methods, and technologies that can be used to control insecticide-resistant cattle fever ticks.

Resolution No. 33
Source: Import-Export Committee
Subject Matter: Embryo Symposium

Resolution

BE IT RESOLVED, that the USDA APHIS sponsor directly or indirectly a symposium, on the international movement of embryos, with research leaders, regulatory officials, and industry to exchange information on current research data and its implications.
Resolution No. 34  
Source: Biologics Committee  
Subject Matter: Animal Biologics Legislation

Resolution  
BE IT RESOLVED that the United States Animal Health Association urges the Congress to enact Section 1923 (the Melcher Amendment, amending the Virus-Serum-Toxin Act of 1913) of S 1714, the Agriculture, Food, Trade and Conservation Act of 1985, the so-called Farm Bill, as it was reported September 30, 1985, by the United States Senate Committee on Agriculture, Nutrition, and Forestry.

PROPOSED AMENDMENT TO USAHA BY-LAWS  
ARTICLE III — MEETING

Friday, November 1, 1985  
Milwaukee, WI

The following amendment is proposed to the By-Laws of the USAHA by deleting the language in lines 291 through 303, and inserting in lieu thereof:

"The annual meeting shall be held at a location and facility determined by the Board of Directors to be adequate for the needs of the membership and in concurrence with the executive officer of the Animal Health Department of the state in which the meeting is proposed.

This selection shall be made at least five (5) years in advance of any annual meeting. The location selected shall be approved by a majority of the Executive Committee."

Moved by Dr. Ragan & seconded by Dr. Goldstein. Passed unanimously at General Session Nov. 1, 1985, Milwaukee WI.
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

Chairman: Dr. W. B. Fairchild, Baton Rouge, LA
Vice Chairman: Dr. K. L. Kuttler, Moscow, ID

J. Lee Alley, AL; R. D. Anderson, NV; J. F. Badger, MO; David M. Bedell, GA; G. M. Brown, IA; G. M. Buening, MO; A. A. Cuthbertson, NV; Chester A. Gipson, MD; R. F. Hall, GA; R. L. Hartin, OK; Thomas J. Holt, MD; J. A. Howarth, CA; Jan D. Huber, NV; M. M. Jochim, CO; E. Wynn Jones, MS; Stuart Lincoln, ID; D. Gene Luther, LA; Marlin L. Main, SD; Michael J. McDonald, KY; W. G. Nelson, ID; J. O. Pearce, Jr., FL; M. Ristic, IL; N. R. Swanson, WY.

Dr. G. M. Buening reported on the prevalence of anaplasmosis in Missouri.

He stated that the objective of his study was to define the present prevalence of anaplasmosis in Missouri beef cattle based on serology. Dr. Buening said the card agglutination test was used to detect serological positive animals.

The results were:
1. Herds sampled 1364
2. Total samples 9800
3. Positive samples 694
4. Negative samples 9106
5. Prevalence rate 7.08%

He said this compared to a prevalence rate of 13% which was found in 1971.

Dr. W. B. Fairchild reported that the prevalence of anaplasmosis in Louisiana cattle was found to be 4.5% in a study that was conducted recently in LA. He said this was a 50% reduction in prevalence from a 1971 survey which found a prevalence of 10%.

The committee discussed the reasons for the reduction of the prevalence of anaplasmosis and suggested that cattle egrets and the increased use of tetracyclines could be responsible for the reduction in the prevalence of anaplasmosis.

Dr. K. L. Kuttler reported on the serologic and clinical responses of premunized, vaccinated and previously infected cattle to challenge exposure to two different Anaplasma marginale isolates.

In summary, two Anaplasma marginale isolates, one originating in Florida and the other from Virginia, were compared immunologically by cross-challenge exposure of 14 anaplasma carrier cattle, eight previously infected cattle, and six splenectomized carrier calves. In addition, 28 cattle vaccinated with a commercially available adjuvant killed vaccine and 22 non-vaccinated cattle were challenge exposed with either the Florida or Virginia isolates.
A detectable clinical response was not produced by either Florida or Virginia isolates when used to challenge carrier and previously infected cattle. However, evidence of *A. marginale* growth as characterized by low percentages of parasitemia and increased serum complement fixation titers was seen in carrier cattle given a heterologous challenge organism and in previously infected cattle inoculated with either homologous or heterologous organisms. Among splenectomized calves, there was virtually no cross protection to the heterologous challenge exposure, whereas a homologous challenge failed to elicit any detectable response.

Vaccinated cattle were resistant to Virginia strain exposure, but the clinical response to Florida strain exposure was severe with a 47% mortality. Most of these cattle displayed typical acute anaplasmosis that was only marginally less severe than that encountered in nonvaccinated cattle.

Dr. Kuttler also reported on preliminary trials using an attenuated *A. marginale* to vaccinate yearling steers. The vaccine strain was modified by deer passage, and produced minimal response in vaccinated cattle. Challenge with virulent field isolates failed to produce any significant response, indicating a high level of vaccine efficacy.

A resolution was adopted by the committee which requested Dr. Kuttler to continue working with the attenuated vaccine to better determine its potential for use in controlling anaplasmosis in the United States.

Dr. E. Wynn Jones reported to the committee that there are encouraging results being obtained using tick cell culture to propagate *A. marginale*.

After Dr. Jones' report the committee was adjourned at 3:00 p.m.
REPORT OF THE COMMITTEE ON ANIMAL WELFARE

Chairman: E. Mickey Stewart, Lincoln, NE
Vice Chairman: Neal Black, S. St. Paul, MN

L. G. Billingsley, CA; Dewey Bond, DC; G. C. Gilley, NH; Bruce H. Ewald, VA; M. W. Fox, DC; Robert Gadd, SD; Ann Gonnerman, MO; Carl Graham, MO; T. M. Gustafson, NE; Frank Hasenauer, CA; Barbara Heffernan, DC; Michele C. Howard, CA; Donald Jones, KS; R. J. Lee, VA; Marlin L. Main, SD; David J. Meisinger, IA; William D. Miller, VA; Ronnie Polen, NJ; D. C. Randall, IA; Robert A. Rice, FL; R. L. Rissler, MD; Grover W. Roberts, CA; J. D. Roswurm, CA; Joe Schmidt, KS; M. S. Silberman, GA; Christine Stevens, DC; W. C. Stewart, MD; Max Van Buskirk, PA; R. M. Wainwright, NY; Nancy E. Wiswall, MD.

The Animal Welfare Committee met October 29, 1985 in the Van Gogh room of the Marc Plaza Hotel in Milwaukee, Wisconsin. Mr. E. Mickey Stewart, Chairman, called the meeting to order at 1:30 p.m. There were 55 committee members and guests present. (The report of the previous meeting were approved, as mailed to each committee member.)

A panel of three individuals from the University of Illinois, spoke on the "Institutional Responsibilities for the Humane Care, Handling, and Use of Animals in Research." Dr. Taylor Bennett spoke to the responsibilities of the attending veterinarian.

Dr. Bennett is responsible for the training of veterinarians as inspectors and investigators for the humane treatment of research animals. Attending veterinarians must be knowledgeable of the laws and regulations regarding animal welfare. They must evaluate the research animals regularly to determine their health and their environment, and establish and maintain disease control measures which keep disease out of the laboratory. Laboratory animals have 24 hour surveillance and are never unattended by laboratory veterinarians.

Dr. Michael Grover, a member of the panel, commented on what the principal investigator can do to improve the welfare of animals. Unnecessary experiments and inflicting unnecessary pain on animals is not condoned. Animals receive drugs prior to surgery as well as after for the relief of pain. The tolerance of pain varies in animals as it does in humans. The principal investigator insures against duplication of experiments involving research animals and strives to receive maximum amount of data at a minimum of cost.

Dr. Karen Hiiemae presented her views as to what the institutional administrator can do to improve the welfare of animals. The administrator must have authority, responsibility and accountability to properly administer any program. The administrator of laboratory research facilities, must educate the public as to the importance of laboratory animals for research, and request and obtain necessary funding to run the program.
This was an excellent panel and their comments were direct and to the point.

Dr. Dick Rissler, Assistant Director of Animal Health Programs, APHIS, discussed enforcement activities of his agency:

1. The number of regions was reduced from 5 to 4.
2. The number of inspections have been increased to an acceptable level (averaging approximately 2.5 investigations per facility).

Dr. Rissler and Cathy Liss told about some of the provisions in the Senate amendment to the Farm Bill which pertained to laboratory animal welfare. Information is sketchy at this time, since the Bill was passed the day before our meeting.

One resolution will be offered to the resolution committee.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

Chairman: Vaughn A. Seaton, Ames, IA
Vice Chairman: Kenneth D. Weide, Columbia, MO

W. F. Alexander, OK; A. A. Andersen, IA; R. P. Azelton, MO; D. E. Bartlett, WI; Joe Bearden, MS; E. A. Carbrey, IA; C. S. Card, PA; Ralph W. Cellon, Jr., FL; R. A. Crandell, TX; G. L. Crenshaw, CA; G. D. Gurss, KS; William T. Harrer, MT; Lenn R. Harrison, GA; N. W. Kruse, NE; G. Lambert, IA; Albert J. Luedke, CO; C. S. McCain, OK; C. A. Mebus, NY; P. A. O'Berry, IA; B. I. Osburn, CA; J. A. Schmitz, NE; R. D. Schultz, WI; L. M. Siegfried, WI; W. L. Sippel, FL; Richard Smith, KS; P. L. Spencer, IL; N. R. Swanson, WY; M. Van der Maaten, IA; Bruce Widger, NY.

The Committee met at 1:35 p.m. in the DaVinci room, October 29, 1985. Members present 15, guests present 20.

The following reports were received by the Committee:
1. Diagnostic Features of Bovine Respiratory Syncytial Virus-Associated Pneumonia of Dairy Calves
2. Acute Bovine Respiratory Syncytial Virus Respiratory Disease in Beef Calves
3. A Comparison of Naturally Occurring and Experimentally Induced Haemophilus somnus Pneumonia in Cattle
4. Is There a Scrapie-Like Disease in Cattle?
5. The Effect of Infectious Bovine Rhinotracheitis Virus on the Bovine Reproductive Tract
6. Use of Antibiotic Additives to Bovine Semen

DIAGNOSTIC FEATURES OF BOVINE RESPIRATORY SYNCYTIAL VIRUS-ASSOCIATED PNEUMONIA OF DAIRY CALVES

Ronald E. Werdin, DVM, PhD*
John C. Baker, DVM, PhD**

Many etiologic agents both viral and bacterial have been incriminated

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in cases of enzootic calf pneumonia throughout the world. In a study performed in 1983 and 1984, it was shown that bovine respiratory syncytial virus (BRSV) was involved in numerous cases of enzootic pneumonia of dairy calves in Minnesota, either alone or in concert with bacteria, primarily Pasteurella spp.

Ages of the calves ranged from two weeks to twelve months. The onset of the disease was rapid with a high rectal temperature, slight nasal discharge, cough and increased respiratory rate. Severe dyspnea was an important and consistent clinical feature. Morbidity approached 100% while mortality ranged from 0–15%.

Macroscopic pulmonary lesions included voluminous, non-collapsing lungs that are reddened in a lobular pattern and are semi-firm and rubbery upon palpation. Interstitial and bullous emphysema were observed frequently. Consolidation of the anterioventral portion of the lung was present when bacterial agents are involved.

Microscopic lesions consisted of both proliferative and necrotizing bronchiolitis. Interstitial pneumonia was observed, often with the presence of syncytial giant cells, some of which contained intracytoplasmic inclusion bodies. When bacterial agents were involved, an exudative pneumonitis was superimposed.

Diagnosis in most cases could be confirmed by a direct fluorescent antibody test performed on frozen sections of lung. Virus isolation has been found to be a less than satisfactory diagnostic tool. Serology utilizing a microtiter serum-virus neutralization test has been useful in herd diagnosis.

ACUTE BOVINE RESPIRATORY SYNCYTIAL VIRAL RESPIRATORY DISEASE IN BEEF CALVES

W. N. Harries, G. A. Chalmers, G. Papp-VID*

Summary

Over the last 10 years in southern Alberta an acute respiratory disease has been observed in beef calves of 3 to 9 months of age. Pathologically, the disease is characterized by an initial degenerative and occlusive bronchiolitis associated with the presence of syncytial giant cells. Secondary pulmonary changes led to a more generalized pneumonia. Histological studies revealed a close resemblance to the changes described for bovine

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respiratory syncytial virus (BRSV) pneumonia in European literature. Serological studies conducted on in-contact calves, and tissue immunofluorescence performed on lung using fluorescein isothiocyanate-conjugated anti-BRSV serum gave added support for this diagnosis. The clinical signs gross and microscopic pathology, and the tissue immunofluorescence techniques were discussed.

A COMPARISON OF NATURALLY OCCURRING AND EXPERIMENTALLY INDUCED *HAEMOPHILUS SOMNUS* PNEUMONIA IN CATTLE

J. J. Andrews, D.V.M., M.S.

Abstract

Although *Haemophilus somnus* (HS) syndrome have been recognized since 1956 it has been only recently that the importance of two other HS syndromes in cattle have been emerging. These syndromes include 1) the respiratory syndrome in young calves (prior to entry into feed lots) characterized by subacute to chronic bronchopneumonia, and 2) reproductive infections of adults characterized by abortions, post parturient endometritis and infertility.

Respiratory infections in young beef and dairy calves may produce significant reduction in growth rates and feed conversion with subsequent economic loss without the pneumonic infection being detected clinically. Likewise, the chronicity of the infection contributes to poor response to antibiotic therapy.

Over the past several years our laboratory has investigated the naturally occurring pneumonia in young calves associated with the isolation of HS and has attempted to reproduce the syndrome in young calves via intratracheal inoculation of viable HS organisms. In addition, we have attempted to improve diagnostic capabilities by comparing the application of diagnostic techniques for detection of HS infections in experimentally infected calves to natural infections.

Naturally occurring HS pneumonia of cattle is characterized by a purulent to necrotizing bronchiolitis and bronchopneumonia often developing into bronchiolitis obliterans and peribronchiolar fibrosis. Many HS infected animals also have a diffuse interstitial pneumonia in the more caudal dorsal lobes with microscopic lesions of interstitial thickening and multifocal hemorrhage.

Experimental infections of young calves with HS reproduced the purulent bronchiolitis, bronchopneumonia, and bronchiolitis obliterans but were unsuccessful in inducing diffuse interstitial changes. Several herd investigations further suggested that naturally occurring HS pneumonia frequently exists with viral infections such as bovine respiratory syncytial virus (BRSV).

The use of serologic methods and HS isolation attempts from the lungs
and nasal passages have also been suggested as techniques for determining the presence of HS in individual animals and in herds.

HS was isolated from the pneumonic lungs of 20 of 28 experimentally infected calves. HS was reisolated from all lungs of calves infected for less than 48 hours and 14 of 22 calves infected for 72 hours or longer. *Pasteurella multocida* (PM) was also isolated from the lungs of 6 of these calves. PM was isolated from 32 of 162 (23%) lungs with naturally occurring HS pneumonia.

PM was isolated from the nasal passages of 20 of 28 experimental calves as well as from 26 of 33 calves from a known HS infected herd. HS was isolated from the nasal passages of only 4 of the 28 experimentally infected calves and none of 33 calves from a naturally infected herd.

Microscopic agglutination titers (MAT) to HS were consistently and markedly higher than complement fixation titers (CFT) in the same animal before and after experimental HS infections.

The complement fixation test for HS appears to be a relatively insensitive method for detecting antibody to HS. The microscopic agglutination test for HS appears to be overly sensitive with titers in the range of 1:64–1:128 and perhaps higher being of questionable significance. The MAT responses of experimentally infected animals were stronger than CFT responses. These results suggest that the use of acute and convalescent sampling is essential to the diagnosis of HS infections when using either CFT or MAT serological methods.

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**IS THERE A SCRAPIE-LIKE DISEASE IN CATTLE?**

R. F. Marsh*, DVM, PhD and G. R. Hartsough+, DVM

Transmissible mink encephalopathy (TME) is a rare disease of ranch-reared mink which is indistinguishable from sheep scrapie. Previous studies on the epidemiology of TME have not identified a definite source of infection for mink. Studies on experimental transmission have shown that mink are susceptible to intracerebral inoculation of American Suffolk scrapie, but that the incubation periods are longer (>1 year) than those observed in natural outbreaks of TME (<1 year).

In April of this year, a mink rancher in Wisconsin reported a debilitating neurologic disease in his herd which we diagnosed as TME by histopathologic findings confirmed by experimental transmission to mink and squirrel monkeys. The rancher was a "dead stock" feeder using mostly (>95%) downer or dead dairy cattle and a few horses. Sheep had never been fed.

We believe that these findings may indicate the presence of a previously

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unrecognized scrapie-like disease in cattle and wish to alert dairy practitioners to this possibility.

THE EFFECT OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS ON THE BOVINE REPRODUCTIVE TRACT

Janice M. Miller, DVM, PhD
and
Martin J. Van Der Maaten, DVM, PhD
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A series of experiments was conducted to investigate the potential role of infectious bovine rhinotracheitis virus (IBRV) as a cause of infertility and early embryonic death in cattle.

Heifers were exposed to IBRV at estrus by intrauterine inoculation. Histopathologic studies showed that although some virus isolates induced a severe, necrotizing endometritis, lesions were limited to the immediate area surrounding the site of virus deposition. The only other significant lesions were found in corpus luteum. In this tissue the virus induced a severe, multifocal necrosis. Similar localized lesions were induced when virus was given by the intramuscular route, while intravenous inoculation resulted in a diffuse, virtually complete, necrosis of the corpus luteum and also necrosis of ovarian follicles.

Using the intravenous route of inoculation, heifers were exposed at estrus to modified-live IBRV. Four strains, representative of those used for vaccine production, were tested and all of them induced the same type of luteal and follicular lesions that characterized field isolates of the virus.

In heifers with extensive luteal necrosis there was a pronounced deficiency of plasma progesterone that was incompatible with continuation of pregnancy in bred heifers. However, except for an occasional delay in returns to estrus, there was no apparent long-term functional impairment of the ovary. Furthermore, when recovered heifers were given corticosteroid, virus was only rarely reactivated and there was no recurrence of ovarian lesions.

To determine the effect of postestrual IBRV infections, bred heifers were inoculated intravenously at 1, 2, 3, or 4 weeks after breeding and necropsied 2 weeks after inoculation. Corpus luteum lesions were seen only in heifers inoculated at 1 or 2 weeks gestation. Three of 4 heifers inoculated at these times had a pregnancy failure. In 1 heifer the cause of embryonic death was shown to be an IBRV infection of the conceptus, as demonstrated by virus isolation, immunofluorescence and electron microscopy.

Antibiotic Additives to Bovine Semen

Dr. Bartlett presented background and historical information concerning the use of antibiotic additives to bovine semen. The current
antibiotic regimen in bovine semen used for artificial insemination has been in effect for about 30 years.

Dr. Lein presented results of studies underway at Cornell University. Dr. Bearden presented a recommendation to the Infectious Disease of Cattle Committee for consideration.

A Recommendation for Approval of the Committee on Infectious Diseases of Cattle, USAHA

In consideration of the recent studies by Drs. Donald H. Lein and Sang Shin of the College of Veterinary Medicine, Cornell University, re-evaluating antibiotic additives for use in processing of bull semen, it is recommended by the Committee of Infectious Diseases of Cattle, USAHA, that a final concentration in each 0.5 ml of frozen semen of:

- Gentamycin 125 mcg
- Lincomycin 150 mcg
- Spectinomycin 75 mcg
- Tylosin 25 mcg

may be used in lieu of the previously recommended final concentration in each 1.0 ml of frozen semen of:

- Polymyxin B sulfate 1000 mcg
- Dihydrostreptomycin 1000 mcg
- Potassium penicillin G 500 I.U.

A motion was made to accept the recommendation and be made a part of this committee’s report. The motion passed.
REPORT OF THE COMMITTEE ON ENVIRONMENTAL RESIDUES

Chairman: G. D. Osweiler, Ames, IA
Vice Chairman: W. B. Bixler, Rockville, MD

John Adams, VA; Fred M. Applehans, TN; David S. Beck, KY; David Bedell, GA; Lee H. Boyd, VA; William B. Buck, IL; S. J. Couger, TX; Larry E. Davis, IL; G. T. Edds, TX; Roland A. Gessert, VA; H. S. Gosser, GA; Carl Graham, MO; J. W. Howder, WA; Lea McGovern Kennedy, VA; Ronald E. Lowe, MD; Jane Robens, MD; D. G. Rollins, MO; P. F. Ross, IA; J. L. Shupe, UT; Richard Stroud, WI; Larry G. Sullivan, MI; T. M. Wilson, PA.

The Environmental Residues Committee met Tuesday, October 29, 1985 at 1:30 p.m. Eleven members and nine guests were present. Reports were presented by seven committee members.

Dr. Wm. B. Bixler, Committee Vice Chairman, reviewed the Activities and Objectives of Surveillance and Compliance in the Center for Veterinary Medicine. Major activity has included development of changes to expedite and speed the review process for animal drug review. Risk assessment activity focuses on risk to humans of compounds found in the food supply which cause cancer in laboratory animals. Additional activities include emphasis on illegal import, distribution and use of veterinary prescription drugs without veterinary supervision. Other current activities include a tissue residue program, nutritional aspects of generic pet foods, and new and improved tissue methodology.

Dr. Lea Kennedy, Division of Animal Feeds, reviewed work done by FDA on milk transfer from aflatoxin contaminated feeds. Both high and low producing cows were studied with high producers having a greater feed to milk ratio of Aflatoxin B1 to Aflatoxin M1.

Recent decisions about use of action levels versus tolerances does not allow full implementation of these results.

Sulfonamide residue considerations were reviewed in light of new regulations about sampling. Representative samples are taken, and animals held while results are obtained on-site. This approach is to counter an approximately two-fold rise in violative residues in recent years.

Propylene glycol in semi-moist pet foods is being reviewed for its potential effects in puppies and cats, especially if those animals are anemic. These studies were requested as a result of review by the newly formed Advisory Committee for the Center for Veterinary Medicine.

Another problem that has recurred recently is the toxicity of imported rawhide chew bones. Dogs exhibit hallucinations and seizures, but the causative agent has not yet been identified.

The EPA National Laboratory Audit Program was reviewed by Dr. Roland Gessert of the Hazard Evaluation Division of EPA. The program
provides for good laboratory practice inspections of industrial or contract laboratories doing pesticide registration studies. In addition, data audits are done in accordance with specified sound methodology. Comments and investigations are used to suggest improvements where needed. Since the program has begun there has been a marked improvement in quality of studies.

Dr. Larry Sullivan, Michigan Department of Agriculture, described a program of disposal of silos contaminated with polychlorinated biphenyls (PCB). Silos coated with PCB’s in the 1960’s and 1970’s were identified in an extensive statewide survey. Of 26,000 silos, 482 were identified as contaminated. In 1985 a dismantling program was begun and such silos are being disposed of in appropriate sites. If contamination is greater than 50 parts per million, the silo must be treated as a hazardous waste. After dismantling of silos, soil removal and replacement completes the cleanup procedure.

Dr. Jane Robens, National Program Staff, USDA, ARS, provided an update on mycotoxin research through ARS. Four centers for research are located in Peoria, Illinois, New Orleans, Louisiana, College Station, Texas, and Athens, Georgia. The Peoria laboratory concentrates on ecology of mycotoxins, analytical methodology, and biosynthetic pathways for mycotoxin formation.

The New Orleans laboratory is working with genetic resistance of corn to fungal invasion and mycotoxin production. Contaminants of grain dust from grain movement are also studied. Most common toxins found are deoxynivalenol (vomitoxin), zearalenol, and secalonic acid.

The Russell Research Center in Athens has recently completed work on the effects of Cyclopiazonic Acid (CPA) as well as reproductive studies and residues in tissues.

At College Station, the most recently established laboratory, activities include density segregation of mycotoxins in contaminated grain, fate of T-2 mycotoxin in rumen fluid, and ultrastructural effects of several mycotoxins in animal tissues.

The Committee discussed priorities for mycotoxin research needs. There was general agreement that T-2 toxin, while well studied experimentally is not found as a field problem.

Reproductive effects of mycotoxins especially vomitoxin in livestock are generally poorly understood and not well researched. Emphasis should still remain on vomitoxin for its effects and occurrence. In addition, the committee recognizes a need for better analytical methodology for mixed feeds or complete feeds.

Dr. Carl Graham, Farmland Industries, reported on mycotoxin related problems in the feed industry. Generally a very low incidence of aflatoxin occurs in their supplies. Questions of feed refusal are occasionally associated with vomitoxin exposure.
Dr. Terry Wilson explained the Fusarium research and reference center at Pennsylvania State University. Several isolates of *Fusarium moniliforme* when fed to rats appear to experimentally be associated with severe hepatocarcinogenicity. This experimental observation should be studied in more detail.

Additional discussion provided by Frank Ross, National Veterinary Services Laboratories, involved explanation of the American Association of Feed Control Official Feedcon Program. It is a data bank of chemical and mycotoxin residues collected and distributed monthly.

Dr. Richard Stroud, National Wildlife Health Laboratory, answered questions about the current status of steel shot replacing lead for migratory waterfowl hunting.

Meeting was adjourned at 4:00 p.m.

Respectfully submitted,

Gary D. Osweiler, Chairman
REPORT OF THE COMMITTEE ON EPIZOOTIC ATTACK

Chairman: Joe Finley, Encinal, TX
Vice Chairman: H. A. McDaniel, Silver Spring, MD

John Adams, VA; J. B. Anderson, TN; R. A. Bankowski, CA; Neal Black, MN; J. L. Blair, VA; W. W. Buisch, MD; Ramsay Burdett, OR; S. J. Couger, TX; R. O. Drummond, TX; A. K. Eugster, TX; W. C. H. Glaze, TX; F. A. Hayes, GA; P. R. Henry, CO; B. R. Heron, CA; Michele C. Howard, CA; J. L. Hyde, MD; Linda L. Logan, NY; E. T. Mallinson, MD; N. L. Meyer, VA; M. A. Mixson, AL, Brig. Gen. T. G. Murnane, TX; J. E. Novy, TX; J. S. Orsborn, CA; B. I. Osburn, CA; H. G. Purchase, MD; T. B. Ryan, NC; E. C. Sharman, MD; M. T. Tillery, MD; Max A. Van Buskirk, PA; Stanley A. Vezey, GA.

The meeting was called to order at 1:30 p.m. in the Van Gogh Room. Over 50 members and guests were present.

Dr. Kenneth Hook reviewed the status of programs to eradicate exotic ticks and bovine babesiosis from Puerto Rico. Further studies are needed to develop procedures and techniques to test cattle for babesiosis. Dr. Hook also expressed a high degree of confidence that National Veterinary Services Laboratories was prepared to diagnose all of the most serious exotic diseases of animals. During subsequent discussions, concern was expressed for development of efficient procedures to diagnose calicivirus and other vesicular diseases of pigs.

Dr. James Novy gave an extensive report on the status of screwworm eradication from Mexico. There was much concern for a recent extensive screwworm outbreak only 140 miles south of Brownsville, Texas. Concern was further heightened by Dr. Novy's conclusion that the screwworms responsible for the outbreak were probably taken from the production plant in Southern Mexico. Several needed biological security measures to prevent workers from taking non-sterilized eggs, larva pupae, or adult flies from the production facility had not been taken even though the evidence the screwworm responsible for the outbreak had come from the plant was most convincing.

Dr. Billy Johnson presented an overview of reorganization within Veterinary Services Headquarters. The total number of personnel will be reduced and some functions will be transferred to the field.

Dr. Zimmerman and Ms. Michelle Howard discussed the threat of Nematodirus battus to U.S. sheep. Ms. Howard was optimistic about developing solutions to the problem posed by this parasite. Surveillance, treatment and diagnostic procedures are needed.

Discussion indicated much has been accomplished in finding better ways to dispose of carcasses and other contaminated material when herds and flocks have to be depopulated due to exotic diseases. However, several problems still remain, namely disposal of feathers, containing aerosols.
developed during grinding, fermenting or composting and educating the public regarding these hazards.

Resolutions were discussed and passed on the following:
1. Model state regulations for zoological animals.
2. Heartwater
3. Identifying cattle imported from Mexico into the U.S.
4. Insecticide resistance in *Boophilus* ticks in Mexico.
5. *Nematodirus battus*
6. *Nematodirus battus*
7. *Nematodirus battus*
The Food Animal Hygiene Committee met on Monday, October 28, 1985 at 1:30 p.m. with eight members and five guests present.

The first topic of discussion was concerning the possible impact of the Sulfa Residue Testing Program on the swine industry. It was generally agreed that the economic impact of this program on the swine industry would be considerable and the group agreed to defer to the State Directors for any resolutions or further comments.

There were comments from the floor concerning a FSIS proposal which may have the effect of requiring State and Federal packing plants to meet U.S. Public Health drinking water standards in their establishments. No resolutions were offered following this discussion.

Dr. Joseph Paige, FDA—Center for Veterinary Medicine, provided comments on activities relating to recent studies on the occurrence of campylobacter and salmonella in food supplies of animal origin. Those present were encouraged to attend Dr. Paige’s formal presentation before the general meeting of the Association.

A general discussion of the current status of testing procedures for trichinosis in swine and horses ensued. No resolutions were offered concerning this topic.

The committee adjourned at 3:30 p.m.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: C. L. Campbell, Tallahassee, FL
Vice Chairman: Ralph C. Knowles, Rehoboth Beach, DE

J. B. Anderson, TN; Jesus Castaneda Garcia, Venezuela; George C. Cilley, NH; LeRoy Coggins, NC; P. M. Eppele, MD; George B. Estes, VA; Chester A. Gipson, MD; John B. Healy, CA; Floyd Jones, Panama; M. J. Kemen, NY; Wayne O. Kester, CO; Michael J. McDonald, KY; William D. Miller, VA; Michael J. Nolan, Washington, DC; S. R. Nusbaum, NJ; M. A. Owen, MA; William E. Pace, FL; Linda Schlater, IA; John Smiley, ME; M. B. Teigland, FL; Charles D. Vail, CO; Thomas E. Walton, CO.

The Committee on Infectious Diseases of Horses convened in Milwaukee, Wisconsin, on October 28, 1985, with some 50 members and visitors in attendance. The committee considered a quite full agenda.

Dr. James Pearson reported that for the past 13 years, the National Veterinary Services Laboratories (NVSL) has been testing samples for the equine encephalitides as a part of the Venezuelan equine encephalitis (VEE) surveillance program. Samples have been submitted primarily from state veterinary diagnostic laboratories; however, samples were also submitted by Veterinary Services (VS), USDA veterinarians, and veterinarians in private practice.

In 1984, there were only 9 positive WEE cases of which 2 were confirmed and the others were presumptive. There were 10 positive EEE cases of which 6 were confirmed. To obtain additional information on incidence, Dr. Theodore Tsai of the Center for Disease Control (CDC), Ft. Collins, Colorado, was contacted, for the summary that he had prepared from other laboratories performing encephalitis testing. CDC reported 110 EEE positive cases in 1984 and 10 positive WEE — for a grand total of 120 EEE and 19 WEE positive cases in 1984.

In 1985, there have been 11 positive WEE cases and 2 positive EEE cases diagnosed at NVSL. CDC has reported 29 EEE and 6 WEE positive cases. The total of CDC and NVSL positive cases is 17 WEE and 31 EEE.

In conclusion, there were the fewest cases of WEE in 1984 and 1985 since testing started. In 1984, there were scattered EEE cases with severe outbreaks in New Jersey and Florida but only a few cases in 1985. Antibody was detected against VEE in a few horses, but there was no evidence of recent infection with the virulent subtype.

Dr. C. A. Gipson reported that of 700,638 equidae tested in FY '85 for equine infectious anemia, 3,152 reactors were disclosed for an infection rate of .45% (see attached map).

He further advised the Committee that in concert with recommendations made by USAHA in 1981 to adopt an individual animal test chart for EIA, USDA had revised its VS Form 10-11 which implements this action.
Some states voiced dissatisfaction with the format used on this form, and Dr. Gipson stated that consideration would be given to constructive revisions prior to its next printing.

The Committee was presented data on a new test for equine infectious anemia which had, upon the previous day, been approved by the AAVLD — namely, the competition enzyme-linked immunosorbent assay (CELISA) test. The following is a summation of the report which projected a production date of May, 1986.

Equine infectious anemia (EIA) is a serious, life-threatening disease that is caused by a lentivirus. The lack of a vaccine or a cure makes present control of the disease difficult. Diagnosis of EIA is the primary means for prevention and control of the disease. The Coggins agar gel immunodiffusion (AGID) test is currently the only federally-approved diagnostic test for EIA. The AGID, however, is time consuming, subjective and has limited sensitivity. A new test is reported which minimizes these problems and correlates well with the AGID.

The competition enzyme-linked immunosorbent assay (CELISA) utilizes peroxidase conjugated P26 viral antigen and a monoclonal antibody to P26 that is bound to the wells. In this simultaneous assay, undiluted horse sera is incubated with the conjugated viral antigen. Anti-P26 antibody in the horse serum competes with the bound anti-P26 monoclonal antibody for the peroxidase-conjugated P26 antigen. After the substrate is added for color development, a positive sample remains colorless while a negative sample appears dark green.

150 NVSL checkset samples were tested at five independent laboratories with the EIA-CELISA. The results obtained by these labs correlated well with NVSL proficiency test results. Ninety NVSL samples and 200 clinical samples were tested in the CELISA at the reporting company's laboratory with 99% correlation to NVSL results and AGID.

The CELISA was as specific and sensitive as the AGID in diagnosing EIA. In addition, the ease of performing and interpreting this assay, together with a short turnover time (3 hours), makes the EIA-CELISA a suitable test and aid for EIA diagnosis.

Committee member, Charles D. Vail, President of the American Association of Equine Practitioners, in refuting for the record a statement which had earlier in the year been made as to the position of his organization on EIA, stated that "AAEP is 100% in support of EIA testing for 'aye and for always,' and that this includes not just myself but the executive board and the administration for at least five years to come." This is so noted.

Dr. Robert H. Whitlock, Chief of the Section of Large Animal Medicine at the University of Pennsylvania School of Veterinary Medicine, presented an update on Potomac Horse Fever and listed research priorities which scientists throughout the country involved with the disease felt should be accorded the matter. This data is published herein.
Resolution Number 25 of the 1984 USAHA Proceedings requested that additional research be directed toward equine viral arteritis. Dr. Gipson reported that during this past year Fort Dodge Laboratories had its vaccine "Arvac" approved for general usage. It was further reported that as of now 36 states were allowing its use without restriction.

Dr. John R. Cole presented data on transmissibility and abortogenic effect of EVA in mares, the summary of which is as follows:

A group of 14 pregnant mares was exposed via contact to 4 mares bred to stallions infected with equine viral arteritis virus. There was a demonstrable febrile response in these 4 donor mares, and in 12 of the 14 pregnant mares. All 18 mares became serologically positive after exposure. Equine viral arteritis virus was isolated from the nasopharynx of 5 of 14 pregnant mares, but not from the 4 donor mares. Ten of the 14 mares aborted, and virus was isolated from fetal specimens or placenta of 8.

Dr. M. J. McDonald, Kentucky State Veterinarian, presented an EVA status update in that state with the following conclusions

1. There has been no clinical occurrence of equine arteritis virus infection since June 1984.
2. There have been no virologically confirmed cases of abortion due to naturally acquired infection by equine arteritis virus in 1984 or 1985.
3. The carrier stallion is of major epidemiologic significance in the perpetuation of this disease.
4. Neither vaccine nor field strains of equine arteritis virus have been shown to induce teratological abnormalities or the carrier state in foals born of EVA-infected or vaccinated mares.
5. The vaccine against EVA is safe and efficacious for stallions as well as mares.
6. The disease can be controlled by vaccination of seronegative stallions and by restricting the breeding of equine arteritis virus-shedding stallions to vaccinated or seropositive mares.
7. There is no indication that any of the horses involved in the 1984 outbreak of EVA in Kentucky have been responsible for spread of the infection to horses in other states or other countries.

Mr. Dennis Senne of the National Veterinary Services Laboratories reported on a standard neutralization test for EVA, a summary of his report being as follows:

A standard microtiter virus neutralization test for equine viral arteritis (EVA) was developed and evaluated. Five laboratories were evaluated on qualitative (positive/negative), quantitative (accuracy of endpoints), and combined qualitative/quantitative results of a 30-serum proficiency test. The average qualitative score was 27 correct out of total of 30, with a range of 24 to 30. The average quantitative score was 23 correct out of a possible 30, with a range of 19 to 27. The average number of samples missed by both qualitative and quantitative determinations was 4, with
a range of 0 to 8. Results correlated well between participating laboratories.

The Committee wishes to commend USDA and the entire veterinary research and diagnostic community on its expeditious efforts in developing needed answers on EVA since this organization last met in Fort Worth in 1984.

General discussion ensued regarding contagious equine metritis; however, no specific action was taken on any of its facets.

Dr. Donald R. Bridgewater, USDA veterinarian, reported on a 1985 outbreak of vesicular stomatitis at the Fort Huachuca Army Post in Arizona in which 42 out of 158 horses (27%) exhibited oral vesicular conditions; however, none of the 25 cattle nor 9 goats associated with them showed any vesicular signs. Dr. Bridgewater stated that the source of VS at the post has not been determined.

In view of the similarity of the lesions caused by this virus and those caused by the virus of foot and mouth disease, the Committee felt that this problem should be further addressed by USDA. A resolution to this effect has been prepared for consideration by this body on Friday.

Mr. H. S. Wright of NVSL presented comparative data on five serologic tests for equine piroplasmosis. His summary follows:

In order to qualify for entry into the United States, all equidae from any part of the world are required to pass an “official” test for piroplasmosis. This has been and currently is the complement-fixation (CF) test as described by Holbrook et al. in the Proceedings of the 75th Annual Meeting of the United States Animal Health Association.

Occasional problems with this CF test have prompted a search for alternate procedures with comparable sensitivity and specificity. Todorovic and Carson describe four varieties of serological tests for the diagnosis of piroplasmosis. They include eight different agglutination tests, the complement-fixation test, the gel precipitation test, and fluorescent antibody tests. At the conclusion of their summary, they state that “None of the tests described in this summary are a perfect diagnostic procedure.” While we believe that this may be true, our study was designed to compare CF test results with results of four other serological procedures that had shown some promise during preliminary testing. The tests to be compared with the CF test were: enzyme-linked immunosassay, indirect fluorescent antibody test, card test, and agar gel diffusion procedure. Over 600 serums from animals of known infection status were tested along with sufficient serum from animals of presumable negative status to give a 94% probability of detecting a 5% difference in test results.

Mr. Lucious Chieves, NVSL, reported an evaluation of an AGID test for dourine.

The following conclusions have been drawn from the results of this limited study.
1. A satisfactory AGID test antigen has been produced and standardized from *T. equiperdum* that is capable of detecting the presence of low and high levels of precipitating antibody.

2. The complement fixation test appears to become positive earlier than the AGID test.

3. Long term studies on animals infected with the live agent were not possible because of the rapid progression of the disease; the persistence of complement fixation antibody could not be determined under these conditions. Under natural conditions infected horses normally live from 6 months to 2 years.

4. Cross reactions were not observed in the AGID test. When serum samples were tested from animals known to be positive to other hemoparasitic, bacterial, and viral diseases — *B. caballi, B. equi, equine metritis, glanders, and equine infectious anemia*.

5. Anticomplementary activity does not interfere with the AGID test.

6. The lyophilized AGID test antigen has a shelf life of at least 3 years when stored at 4°C. CF test antigen must be stored at −70°C.

7. The performance of the AGID test does not place any great technical demand upon laboratory personnel.

8. The AGID test could be used to clarify the status of AC samples or samples where the history of the donor animal or other extenuating circumstances provides reason to doubt the validity of CF test results.

9. **NOTE:** The CF and AGID tests were 100% in agreement when 150 known negative samples were tested.

Dr. K. L. Kuttler from the Hemoparasitic Diseases Research Unit of Washington State University, reported on a clinical and comparative serologic response to induced *Babesia equi* infection in mature horses. His summary:

Nine 4-year-old, Arabian geldings were infected with *Babesia equi* of European origin. All horses developed a detectable parasitemia an average of 30 days after inoculation, which was accompanied by a reduction in PCV. No deaths occurred and the infections were generally mild; however, all horses became serologically positive on an average of 30 days post inoculation (PI) with the complement fixation test (CF), and 23 days PI with the indirect fluorescent antibody test (IFA). A significant correlation in CF and IRA titers was detectable. No CF response was seen with *B. caballi* antigens.

The foregoing presentation is an interim report on some of the aspects of a protocol on Babesia trials which was presented to and endorsed by this Committee in 1983.

The Committee expressed grave concern relating to the importation of EP infected horses into the United States and to this end has prepared a resolution for consideration by the membership of this Association on Friday.
In 1982 a subcommittee was appointed to define equine salmonella problems and in its continuing work proposed a resolution in which the Committee concurred that has been submitted for approval by the Association.
Potomac Horse Fever (PHF) is a disease of horses of all ages which was first recognized in Montgomery County, Maryland, in 1979. Affected horses usually develop a high fever (102–107°F), become depressed, eat less which is often followed by a severe life-threatening diarrhea and/or colic; laminitis is a frequent sequel, often necessitating euthanasia. The disease has also been referred to as Potomac Valley Fever and Acute Equine Diarrhea Syndrome (AEDS), among other names. In Maryland alone between 1982 and 1984 there have been 338 reported clinical cases, with 88 deaths. The disease is distinctly seasonal, with most cases occurring between May and November. During this same three-year period approximately 73% of cases have occurred during the months of July and August.

In September, 1983, research at the University of Pennsylvania, done in collaboration with Dr. Allen Jenny of the National Veterinary Services Laboratory, Ames, Iowa, showed that the disease could be transmitted with blood from an infected Maryland horse, whereas diarrheic feces posed little risk to susceptible horses. In March 1984, based on a study done in Dr. Ristic’s laboratory at the University of Illinois, Dr. Jenny reported serologic evidence of a rickettsial agent in recovered horses. In September of that year, researchers at the University of Illinois and Virginia Tech and State University independently reported the identification of a rickettsial organism as the cause of Potomac Horse Fever. Subsequently the findings of the above two groups were confirmed by workers at the University of Maryland and at the University of Pennsylvania. The organism has subsequently been named Ehrlichia risticii in honor of Dr. Miodrag Ristic, of the University of Illinois.

The disease, once thought to be confined to the Potomac River valley of Maryland and Virginia, is known to occur in many regions of the United States. The characteristic epidemiological pattern and the typical clinical signs of the disease have been reported by veterinary practitioners throughout the United States. PHF has been confirmed in at least 15 states including Ohio, Pennsylvania, West Virginia, Virginia, Maryland, New Jersey, Illinois, New York, Wisconsin, Idaho, Minnesota, Kentucky,
Connecticut, Florida, California and Canada by the isolation of the causative organism or the demonstration of specific antibody in affected or recovered horses.

The epidemiology of PHF is unique. Most cases usually occur near the proximity of a large river and its tributaries. The disease is not transmitted directly from horse to horse (which helps differentiate it from salmonellosis, one of the most commonly recognized causes of diarrhea in adults horses) and feces from infected horses are not thought to be infective.\textsuperscript{10} Outbreaks on some farms resembling a contagious disease have been reported but are probably due to simultaneous exposure, not spread from horse to horse. The seasonal nature of the disease, and the proven involvement of ticks in the transmission of some of the other ehrlichial diseases of animals strongly suggests the involvement of an arthropod vector.\textsuperscript{11} However, despite research efforts by the Agricultural Research Service of the USDA at Beltsville, the Virginia-Maryland Regional College of Veterinary Medicine at Blacksburg, the University of Maryland-College Park campus, and at the National Institute for Allergy and Infectious Diseases Rocky Mountain Laboratories in Montana, the vector(s) that transmit the causative agent, \textit{Ehrlichia risticii} has not so far been proven, although ticks and specifically the American dog tick are the primary suspects.

The disease is characterized by a marked variation in the presentation and severity of clinical signs.\textsuperscript{23} The typical case begins with mild depression and anorexia. The horse is usually febrile (102–107°F) and may have injected mucous membranes and prominent scleral blood vessels. Intestinal gas (borborygmal) sounds will be noticeably decreased or absent in the early stages of the disease. In 24 to 48 hours the horse often develops profuse, watery diarrhea which may continue for 10 days but in most cases lasts only 3–5 days. Signs of laminitis, if they occur, begin most commonly after the onset of diarrhea, or rarely before the occurrence of diarrhea. In 1983, laminitis was clinically significant in 25\% of the cases, often resulting in euthanasia for humane considerations.\textsuperscript{7} In 1984, 42\% of the deaths were attributed to founder and its complications. Since the clinical signs strongly resemble salmonellosis, multiple samples of fecal matter should be submitted to a laboratory for culture.\textsuperscript{19,22} If all samples are negative for salmonella then Potomac Horse Fever should be given serious consideration as one of several other causes of acute diarrhea in horses.

Although the majority of cases follow the typical course described, a few do not. Some have transient depression and fever as the only signs. Some cases are febrile (increased temperature), have decreased borborygmal (intestinal) sounds, very injected mucous membranes, abdominal distention and severe abdominal pain (colic). The pain may be so severe as to resemble a horse requiring surgical correction due to an intestinal obstruction. Rarely horses may die before diarrhea develops. The most consistent signs in all cases are depression, fever, and decrease in borborygmal (intestinal) sounds.\textsuperscript{23}
With knowledge that PHF is caused by an ehrlichia, a member of the rickettsial family of organisms, tetracycline is considered to be the antibiotic of choice, since most rickettsial disease responds well to the tetracycline. However, several facts need to be seriously considered before tetracycline is used in treating horses with suspected PHF: 1) Some horses develop diarrhea following the use of tetracycline, especially those that are concurrent salmonella carriers (5–15% of normal horses may harbor salmonella organisms unknowingly); and 2) Tetracycline will not prevent the infection in horses subsequently given infective organisms, the antibiotic merely delays the onset of signs for up to 20 days. The use of intravenous fluids to restore the blood volume correcting dehydration is strongly recommended. Other therapeutic measures depend on the clinical signs shown by the individual horse. Early recognition of signs of illness and seeking veterinary assistance should substantially lessen the mortality. The attending veterinarian is in the best position to recommend and prescribe therapy.

METHODS OF DIAGNOSIS
Currently the best way to confirm the presence of the disease is to identify serum antibodies in paired serum samples to the causative agent *E. risticii* from the acute and recovered horses by the indirect fluorescent antibody test (IFA). The serum samples should be taken at least 2 weeks, and preferably 3 weeks, apart. This test is currently available at:

1) Virginia Tech and State University — Contact: Dr. Y. Rikihisa — Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061 (703-961-4806)

2) University of Illinois — Contact: Dr. Jean Sessions as serologic coordinator for Dr. M. Ristic (Dr. Sessions: 301-963-4664)

3) University of Maryland — Contact: Dr. S. Dutta — Department of Medicine, University of Maryland, College Park, MD 20740 (301-454-6329)

A recently developed ELISA test offers some advantages over the IFA test which requires a cell line with infective organisms on a glass slide, a highly skilled technician and expensive fluorescent microscopy equipment. The ELISA test offers the advantage of completing a large number of samples in a day and should be more reproducible than the IFA test.

During the acute phase of the disease the practitioner has a limited means to make a definitive diagnosis. Blood taken from a clinical patient could be injected into a susceptible research pony, however, it would be 10–16 days before the pony would show typical signs if the suspect animal was positive. Attempts to demonstrate the causative agent in the peripheral blood monocytes is rarely possible using stains to identify rickettsia; for example, acridine orange or giemsa stains and high power (1,000–1,500 X) examination of the buffy coat cells is required.

One promising diagnostic technique is to harvest peripheral blood aseptically, separate the macrophages on a density gradient, and grow
them in tissue culture media which allows the organism \textit{E. risticii} to proliferate in the macrophages enhancing their ability to be detected following staining with acridine orange or giemsa stain.\textsuperscript{9} Another potential diagnostic test is the intraperitoneal injection of blood or buffy coat cells from a clinical case into mice. Several strains of mice appear susceptible including Swiss Webster ICR strain, BalbC and Sprague-Dawley.\textsuperscript{6} Similar to susceptible ponies, the mice begin to show clinical signs on day 10–16 post inoculation which consist of rough hair coat, huddling in the corner of the cage and diarrhea.\textsuperscript{6,8} Thus, due to the time course of events a laboratory animal as the test vehicle provides little diagnostic advantage for the practitioner.

Another but yet unproven diagnostic test is direct fluorescent antibody to detect the organism in peripheral blood monocytes. This diagnostic approach is being pursued, but at this time has not been adequately developed.

As with most infectious diseases, prevention is the best policy. Currently no vaccine or protocol is available to prevent PHF. However, owners are advised to record daily temperatures, appetite and activity. Any variance from normal for that horse should be noted and brought to the attention of the attending veterinarian. Modest evidence exists to suggest recovered horses are immune for more than a year. Although recovered horses have been shown to harbor the organism for extended periods, it is uncertain if they can transmit the disease to other horses.

Since epidemiologic evidence suggests an arthropod vector, the routine use of “approved” pesticides to suppress insect and tick blood feeding is strongly recommended to minimize the risk of Potomac Horse Fever.

Experimental studies revealed that dogs and sub-human primates are susceptible to infection with \textit{E. risticii}. Consequently dogs and possibly other companion animals may play a role in transmission of \textit{E. risticii}. The susceptibility of monkeys to infection with the agent indicates a potential public health hazard.

Research is actively being pursued at several institutions to help provide more answers about this newly discovered disease of horses.\textsuperscript{1,3,14,23} The Morris Animal Foundation, 45 Inverness Drive East, Englewood, CO 80112, (303) 790-2345, under the volunteer leadership of Ms. Sandie Cafritz, as Chairman of the Potomac Horse Fever Committee — Morris Animal Foundation, has provided much of the impetus to obtain funding for this newly recognized disease of horses.

Nine investigators actively working on Potomac Horse Fever were asked to rank/prioritize each specific objective that had been previously identified by the same investigators as fruitful areas of research. The results of that ranking were as follows:
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<th>Ranking/ Priority Score</th>
<th>Specific Research Objective</th>
<th>Ranking/ Priority Score</th>
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<td>Highest 1</td>
<td>Development of a sensitive diagnostic test for the acutely infected horse</td>
<td>1</td>
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<td>2</td>
<td>Determine vectors involved in the transmission of PHF</td>
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<td>3</td>
<td>Develop an efficacious therapeutic regime for practitioners to use on horses infected with PHF</td>
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<td>4</td>
<td>Development of an inactivated or subunit vaccine</td>
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<td>5</td>
<td>Determine if immunity is sterile following infection (Does a carrier state exist?)</td>
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<td>6</td>
<td>Determine duration of immunity following infection following recovery from natural disease</td>
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<td>7</td>
<td>Determine the pathogenesis of PHF which would include the relationship of the organism to the development of lesions</td>
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<tr>
<td>Lowest 8</td>
<td>Study ehrlichial antibody profiles on infected and non-infected forms to determine antibody level needed for protection</td>
<td>8</td>
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</table>

Similarly, practicing veterinarians from Montgomery County, Maryland, also ranked the objectives, the results of which are presented on the right side of the above table.

**REFERENCES**


EQUINE VIRAL ARTERITIS: A STANDARD PROCEDURE FOR THE VIRUS NEUTRALIZATION TEST AND COMPARISON OF RESULTS OF A PROFICIENCY TEST PERFORMED AT FIVE LABORATORIES

D. A. Senne, J. E. Pearson and E. A. Carbrey
National Veterinary Services Laboratories
P.O. Box 844, Ames, Iowa 50010

SUMMARY

A standard microtiter virus neutralization test for equine viral arteritis (EVA) was developed and evaluated. Five laboratories were evaluated on qualitative (positive/negative), quantitative (accuracy of endpoints), and combined qualitative/quantitative results of a 30-serum proficiency test. The average qualitative score was 27 correct out of a total of 30, with a range of 24 to 30. The average quantitative score was 23 correct out of a possible 30, with a range of 19 to 27. The average number of samples missed by both qualitative and quantitative determinations was 4, with a range of 0 to 8. Results correlated well between participating laboratories.

INTRODUCTION

Equine viral arteritis (EVA) is a viral disease of horses which can occur as a clinical or subclinical infection. Typically, clinical signs include fever, anorexia, depression, conjunctivitis, palpebral and leg edema, nasal discharge, congestion of the nasal mucosa, coughing, respiratory distress and diarrhea. Stallions that have clinically recovered from natural infections of EVA have been known to shed virus in semen for several months, thus transmitting the disease to susceptible mares during breeding. In pregnant mares, the disease can be abortifacient.

The recent occurrence of EVA among Thoroughbred horses in Kentucky has stimulated a renewed interest in the disease and provided a new challenge to the diagnostic laboratories. Disease control efforts exerted by the equine industry and the Kentucky Board of Agriculture have centered around a vaccination program using a modified-live virus vaccine. This program requires that horses bred in the state of Kentucky be tested for EVA antibody prior to breeding or vaccination and, in some instances, retested following vaccination. In addition, most countries importing horses from the United States require a negative serologic test for EVA as a health requirement. These requirements have placed a tremendous burden on laboratories performing serologic tests for EVA. Presently, the most sensitive and widely used serologic procedure for the detection of antibody to EVA is the virus neutralization test. Uniformity of results between laboratories has been critical for disease control and protection of our livestock industry, as well as keeping export markets open.
In this report we will describe the currently accepted, standard EVA serologic procedure and evaluate the results of a proficiency test performed by 5 laboratories.

MATERIALS AND METHODS

Serums

A 30-serum proficiency test was prepared at the National Veterinary Services Laboratories (NVSL) using serums submitted from several of the participating laboratories so as to include samples from different geographical areas. The serums were from experimentally and naturally infected horses. Seven of the 30 serums, serums 4 through 10, were prepared by making dilutions of positive, high titered serums in negative horse serum. Samples 4 through 6 were prepared by making serial two-fold dilutions of a single serum. Likewise, samples 7 through 10 were serial two-fold dilutions of a second serum. The 23 remaining samples were undiluted. The serums were bottled as 0.5 ml aliquots, frozen and sent to the participating laboratories.

Virus

A low passage of the Bucyrus strain of EVA virus was supplied for the proficiency test. The virus was propagated in rabbit kidney-13 (RK-13) cells, bottled and frozen at -70 C. Each laboratory was provided with sufficient virus to accomplish standardization and testing of the proficiency test serums without the need for additional passages.

Cell Cultures

Flasks of RK-13 cells were provided to each laboratory. RK-13 cultures were propagated using Eagle's F-15 (Gibco catalog #4101500)* medium containing 10% fetal bovine serum and antibiotics. Cells were routinely passaged at weekly intervals using a split ratio of 1:3.

Test Procedure

1. Inactivate serum at 56 C for 30 minutes.
2. Designate duplicate rows of wells for each serum to be tested.
3. Dispense 0.025 ml of Eagle's F-15 medium containing antibiotics in all wells except the last well of each row which is used as a serum control.
4. Dispense 0.025 ml of undiluted serum into the first well of each duplicate row and also into the serum control well. Note: A micropipetting device should be used to dispense the undiluted serum to the plates because significant errors may result if undiluted serum is transferred using a microtiter loop.
5. Beginning with the first well, serially dilute each serum. Discard the carry-over from the last well. The serum control is not diluted.
6. Prepare a dilution of virus containing 100–300 TCID₅₀ per 0.025 ml. The virus diluent used is Eagle's F-15 medium containing

*Grand Island Biological Company, 3175 Staley Road, Grand Island, NY 14072
antibiotics and fresh guinea pig complement at a final concentration of 10%. Dispense 0.025 ml of the virus-complement mixture to each well excluding the serum control wells. A 0.025 ml aliquot of medium containing complement is added to the serum control instead of virus.

7. Virus controls must be included to assure the validity of the test results. Beginning with the virus dilution calculated to contain 100–300 TDIC\textsubscript{50}, prepare four tenfold dilutions in tubes and transfer to the wells in the microtiter plate. Four wells are used for each virus dilution. Each well contains 0.025 ml Eagle’s F-15 medium, 0.025 ml of the appropriate virus dilution containing complement and 0.1 ml cells. The virus titration must detect 100–300 TCID\textsubscript{50} per 0.025 ml for the test to be considered valid.

8. Agitate plates to insure thorough mixing of serum and virus. Cover and incubate for one hour at 37 C in a humidified 5% CO\textsubscript{2} atmosphere.

9. Remove cells from flasks (75 cm\textsuperscript{2}) of 3- to 5-day-old RK-13 cell cultures using Saline A-trypsin-versene (ATV) or its equivalent. Centrifuge trypsinized cells at 150 x g for 10 minutes and re-suspend the packed cells in Eagle’s F-15 medium supplemented with 10% fetal bovine serum and antibiotics. Use a concentration of cells that will result in a confluent cell monolayer in the microtiter plate wells within 18–24 hours post seeding. Usually 20–30 ml of media can be used for each flash of cells. A reduced virus titer may be observed in the test if the cells are older than 5 days.

10. Dispense 0.1 ml of cell suspension to all wells. Tape plates or cover with plate lids and incubate at 37 C in a humidified 5% CO\textsubscript{2} atmosphere.

11. The test is read for cytopathic effect (CPE) after 48–72 hours of incubation. Serum controls are observed to detect possible toxicity at the lowest dilutions of serum. Estimate and record the percentage of CPE observed in each of the test wells as compared to the degree of CPE observed in the wells of the lowest virus control dilution. A convenient recording system would be as follows: 0 = no CPE; 1 = 25%; 2 = 50%; 3 = 75%; 4 = 100% CPE.

**INTERPRETATION OF TEST RESULTS**

A serum dilution is considered positive if the amount of CPE in both wells of the same serum dilution is 0–25% (75% reduction in the CPE of the virus), when compared to the amount of CPE in the lowest dilution of the virus control wells. Because of the nature of this test, endpoints are subjective and may be difficult to reproduce since partial neutralization may be observed over a range of several dilutions. For instance, consider the following example of the readings obtained different dilutions of a serum:
The endpoint titer of this titration is reported as 1:64 since it is the highest dilution with at least 75% reduction in CPE. All titers are reported as the final serum dilution prior to addition of the cell culture suspension. A serum initially diluted 1:2 would be a 1:4 dilution following the addition of virus.

**EVALUATION OF PROFICIENCY TEST RESULTS**

The expected result for each test was established by using the median value findings reported by the 5 laboratories. Results obtained from each laboratory were evaluated by 3 criteria: positive or negative (qualitative), accuracy of endpoints for positive serums (quantitative) and the combined qualitative/quantitative score.

A maximum qualitative score of 1 was assigned to each result which was in agreement with the expected result of positive or negative. A qualitative score of zero was assigned to results which disagreed with expected results. A maximum qualitative score of 30 would indicate complete agreement with the expected results for all samples.

Quantitative scores were likewise assigned a maximum value of 1 if the reported endpoint was in agreement with the expected result. A value of 0.25 was deducted for each two-fold difference in the reported endpoint, as compared to the expected endpoint. A maximum quantitative score of 30 would indicate complete agreement with expected results on all samples.

The criteria for the combined score was based on the number of samples missed using positive/negative and endpoint differences of greater than 8-fold on positive samples. Most differences between laboratory findings and the median values of positive samples were 8-fold or less.

**RESULTS**

The standard microtiter virus neutralization test procedure was developed as a pool of techniques used by the participating laboratories. At the time of the evaluation, the laboratories were currently performing serological assays for EVA on a routine basis and were familiar with the problems associated with test interpretation. Each laboratory was requested to provide the results of the proficiency test using the standard procedure, and if they wished, results obtained using an alternate procedure.

Proficiency test results obtained by each participating laboratory using the standard test procedure are presented in Table 1. Each laboratory’s numerical scores are presented in Table 2.

The numerical scores of qualitative results ranged from 24 to 30 with an average of 27 correct results out of a total of 30 samples. The quantitative results were more variable with a range of 19 to 27 and an average of 23 correct results out of 30. The average number of results which disagreed with the expected results based on the combined qualitative/quantitative score, was 4, with a range of 0 to 8.
DISCUSSION

Until recently, the need for a standardized EVA serologic procedure was not of real concern among laboratories performing these tests. With the occurrence of the disease among Thoroughbreds in Kentucky and the subsequent measures taken to control the disease, the need for standardization has become increasingly important. Because interpreting test results is subjective, there has been increased interest among laboratories to have a proficiency test for use in evaluating the sensitivity and reproducibility of the test system.

The most important aspect in performing EVA serology involves the determination of sero-positive and sero-negative horses. Although endpoint determination on paired serums can be of significant diagnostic importance, positive/negative results are of greater importance for purposes of exporting horses and in satisfying breeding requirements. In this category, the results of the proficiency test results appear to be quite good. The average agreement of positive/negative samples was 27 out of 30 samples.

Although the number of variables involved in this comparison was reduced by supplying all laboratories with virus and cell cultures, the source of complement used in the test was left to the discretion of each laboratory. Whether differences in the source and potency of complement would have affected test results is not known. The effect of complement on the test certainly needs further evaluation.

Diluted serums were used in the proficiency test to evaluate the consistency of results obtained at each laboratory. All laboratories reported results consistent with the serum dilution on these samples, indicating a high degree of accuracy in conducting the test.

The proficiency test has established a basis for evaluating laboratories performing EVA serologic tests. The results presented in this paper verify the reliability of this procedure.

ACKNOWLEDGEMENTS

The authors would like to thank the following laboratories that made this evaluation possible: Diagnostic Laboratory, New York State Veterinary College, Cornell University, Ithaca, NY; Florida Animal Disease Diagnostic Laboratory, Kissimmee, FL; University of Kentucky Livestock Disease Diagnostic Center, Lexington, KY; Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Colorado State University, Fort Collins, CO.

A special thanks to Dr. Merrill Swanson for assistance in the statistical analysis of the results.

REFERENCES

### Table 1. Test results of 5 laboratories on 30 serums

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### Table 2. Laboratory scores on proficiency test

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REPORT OF THE COMMITTEE ON PHARMACEUTICALS, PESTICIDES AND RELATED TOXICOLOGY

Chairman: W. A. Knapp, Jr., Raleigh, NC
Vice Chairman: G. D. Lindsey, Indianapolis, IN

D. A. Armstrong, NE; D. T. Bechtol, TX; Robert R. Biddle, DC; W. B. Bixler, VA; William B. Buck, IL; A. A. Chadwick, DE; M. L. Crandall, MD; L. M. Crawford, MD; G. T. Edds, TX; D. O. Farrington, IN; J. E. Fox, GA; D. A. Gable, VA; R. A. Gessert, VA; Robert L. Gillespie, MD; J. S. Gloyd, IL; J. S. Hayden, MO; Morris R. Levy, MD; Vader M. Loomis, MD; D. R. Mackey, CO; Larry F. Moore, KS; Gary D. Osweiler, IA; M. G. Scroggs, OK; T. K. Shotwell, TX; T. P. Siburt, VA; Marty Vanier, VA; D. L. Wilkes, CO.

The Committee on Pharmaceuticals, Pesticides and Related Toxicology met on Tuesday afternoon, October 29, 1985, 1:30-5:00 p.m. Fifteen members and nine guests were present for a total of twenty-four in attendance.

There were several important program subjects consisting of formal presentations and extensive discussion periods. All were of special interest to practicing veterinarians, the veterinary profession, and other interested groups and organizations.

The first subject to be discussed was the Center of Veterinary Medicine's (CVM's) Bulk Drug Proposal. This proposal, in effect, would allow a drug manufacturer, and the holder of an NADA for a bulk drug, to sell that drug to a veterinarian who could, in turn, prepare a final formulation with that drug by following specific instructions supplied by the manufacturer. The final formulation (powder, capsule, tablet, ointment, injectable solution etc.) could be used by the veterinarian as a prescription (Rx) only drug in his/her own practice for the prevention and/or treatment of diseases of animals with which the practicing veterinarian has a bona fide client/patient relationship. The participating veterinarian would not be subject to Good Manufacturing Practice (GMP) regulations, or periodic FDA inspections according to the proposal as presently written. Only drugs with (FDA) approved NADA's and pre-1962 drugs would be available to veterinarians under this proposal. Max L. Crandall, DVM, Center of Veterinary Medicine made the presentation.

The discussion that followed was extensive. There was much diversity of opinion and no consensus. There appeared to be more negative than positive opinions within the committee membership regarding the Bulk Drug Proposal. Much of the concern focused upon the lack of provisions for identification of and accountability by veterinarians or pharmacists who would manufacture finished dosage forms from bulk drug substances pursuant to the proposal. Such is in sharp contrast to the high level of accountability currently required by manufacturers of finished animal drugs.
It is important that every interested person become familiar with the Bulk Drug Proposal and submit written comments to the Center for Veterinary Medicine by November 14, 1985. Your comments are extremely important and earnestly solicited.

The second program agenda topic was a status report on the illegal importation, sale, distribution, and use of animal drugs and drug substances by William B. Bixler, VMD, Center for Veterinary Medicine.

FDA recognizes only two legal categories of veterinary drugs, OTC and Prescription (Rx) drugs. When prescription drugs are used, a recognized veterinarian-client-patient relationship must exist. The Center for Veterinary Medicine (FDA) is soon to have a brochure available for distribution entitled “FDA Regulation of Veterinary Prescription Drugs.” A copy may be requested through CVM.

The discussion that followed indicated that the illegal importation, sale, distribution and use of animal drug substances and finished drugs are indeed prevalent, serious, and increasing. This problem has not been adequately addressed either by veterinarians or the veterinary profession. This is regrettable. The longer remedial action is postponed the worse the problem becomes.

The third topic was addressed by Max L. Crandall, DVM Center of Veterinary Medicine. It has been proposed that all injectable antibiotics be available only on the prescription of a veterinarian. While there is considerable rationale to support such a proposal, committee response was quite mixed. It was apparent that much more discussion, in professional and public forums, should be scheduled before this issue is satisfactorily resolved.

The Committee wishes to thank CVM and Doctors Bixler and Crandall for supporting the work of our committee and for presentations on these important subjects.

The fourth and last topic was presented by Dr. Larry Thompson of the National Animal Poison Control Center located at the University of Illinois who outlined a proposal for the development of a nationwide network of regional and state laboratories with a national center to coordinate an overall program for emergency preparedness on chemical disasters in animals. This program called, National Animal Poison Information Network (NAPINet) would distribute the monitoring for and management of animals poisoning and chemical contamination problems in animals and expedite a response to those problems.
REPORT ON THE COMMITTEE ON PROFESSIONAL OVERSIGHT

Chairman: P. L. Smith, Sacramento, CA
Vice Chairman: H. E. Golstein, Columbus, OH

C. L. Campbell, FL; J. C. Jefferies, FL; J. L. O'Hara, AZ; J. O. Pearce, Jr., FL; J. R. Ragan, TN; S. T. Wilson, Jr., D.C.

The USAHA Committee on Professional Oversight met at 1:30 p.m. on Thursday, October 31, 1985; all members were present.

Two topics were discussed that resulted in Committee recommendations.

1. The National Assembly of Chief Livestock Health Officials expressed concern of the intended use of a new small animal health certificate which has been developed by APHIS. The Committee recommends that APHIS clarify to the State Veterinarians how this certificate is intended to be used. It was further recommended that if this certificate is used for interstate shipments, that copies be distributed in a manner compatible with present procedures for large animal certificates.

2. The Committee along with the National Assembly of Chief Livestock Health Officials is concerned about procedures used in accreditation violations. Aggressive action on flagrant violations is supported and needed. The concern is that there will be an over reaction in the area of minor violations. The Committee recommends that the accreditation program and violation considerations remain a state-federal cooperative program. It further recommends that discretionary power must be allowed to the Veterinarians in Charge and the State Veterinarians in the handling of minor accreditation violations.

Four resolutions were considered by the Committee. Three were approved and presented to the Resolutions Committee for consideration. The resolutions were on the subjects of cattle fever tick treatment procedures on imported Mexican cattle, accreditation of attending veterinarians, and the management of slaughter and livestock exposed to rabies.
REPORT OF THE COMMITTEE
ON STATE FEDERAL RELATIONS

Chairman: N. W. Kruse, Lincoln, NE

Philip E. Bradshaw, IL; Ramsay G. Burdett, OR; J. S. Cobb, GA; H. E. Goldstein, OH; John W. Holcombe, TX; J. F. Hudelson, CO; V. P. LaBranche, MA; J. O. Pearce, Jr., FL; J. C. Shook, MD; David U. Walker, VT.

The State-Federal Relations Committee met with representatives of Animal and Plant Health Inspection Service, Agricultural Research Service, Center for Veterinary Medicine, Food Safety Inspection Service and Agency of Transportation receiving an update on FY 1986 priorities together with a directional analysis for FY 1985. This Committee applauds all representatives for efforts expended in program preparation and sharing of policy decisions both budgetary and program oriented. Presentations were well organized, factual and most informative. Based on actions taken at the USAHA Annual Meeting, this organization will support the predominance of USDA program policy.

In general, committee members were appreciative of responses to the various USAHA resolutions adopted at the 1984 Annual Meeting, however, concerns remain in some specific areas which will be expressed in the remainder of this report. Those areas of basic agreement will be given less attention.

This committee wishes to emphasize a consensus that state government, federal government and agricultural industry must participate equally in development and implementation of livestock disease control programs. USAHA is respected worldwide as an effective interface between state government, federal government and agricultural industry leading to effective, workable programs. Hopefully, these structured representations will continue to benefit the people of this country through a powerful and healthy livestock industry. Continued extensive federal participation and representation is greatly needed in USAHA.

This committee reiterates its request for independent review of the concept of Regional Offices—Veterinary Services—APHIS as expressed in the 1984 STATE-FEDERAL RELATIONS COMMITTEE REPORT. We do appreciate the in-house review undertaken in the past year, but suggest that an independent evaluation could result in a more efficient, cost-effective approach to regional management.

1. BRUCELLOSIS BUDGET—This committee was most encouraged by FY 1984–1985 brucellosis program statistics presented. Nationally, this program is viewed as crucial to credibility of livestock disease control programs in the United States. We were particularly discouraged by APHIS’s proposed FY 1986 budgetary dismantlement of this valuable program. Risk of widespread reinfection with this fulminating disease would revert from a statistical probability to a bonafide fact. Therefore,
this committee strongly recommends that brucellosis eradication funding be restored in such a manner that federal participation will be equal to the level of contribution necessary in personnel, material and program services, including indemnity, to equal that of FY 1985. This committee maintains adamant opposition to dependency on user fees to support a program of such vital interest to the health and welfare of this nation.

2. SCREWWORM BUDGET — We urge continuance of the screwworm program in Mexico and commend APHIS on progress made. We strongly recommend that funds be provided to continue this program at the present level.

Studies should be conducted to determine if it is feasible to continue this program until the eradication zone extends through Panama. This extension could be most cost-effective over the next several years.

3. ANIMAL WELFARE BUDGET — The Committee must record a very strong opposition against the total deletion of funds for Animal Welfare. We believe the United States Department of Agriculture is the correct agency to administer this important part of the interests of USAHA.

4. POULTRY — We commend veterinary services for the manner that Avian Influenza was contained and eradicated. We, also, approve in the continued surveillance for this disease. The committee endorses a continued study so that any Task Force operations of this sort in the future may profit from the experiences of the Avian Influenza effort. This committee recommends continuing research into the pathogenicity of Avian Influenza.

This committee recommends that APHIS initiate a program that would require licensing of dealers of exotic birds who deal interstate. These dealers would be required to keep records of all purchases and sales and possibly leg band or other identification on some species.

We feel that VVND poses too great a threat to our Poultry Industry to continue allowing unscrupulous smugglers and dealers to pose the present threat to this industry.

5. TUBERCULOSIS HERD DEPOPULATION — We recommend budget allocations remain to permit funding to support depopulation of infected herds and exposed animals that had moved from infected herds.

We urge an increase in the submission and improvement in the identification of slaughter samples to aid in the identifying and eradicating tuberculosis. Immediate quarantine of infected herds and testing of adjacent herds is of utmost importance in our eradication program.

TB—MEXICAN CATTLE — We recommend continued research to determine methods to properly identify imported Mexican feeder steers to contain the spread of tuberculosis in our feedlots.

TB—AMERICAN BISON — Whereas tuberculosis is an apparent problem in this species, inclusion of bison in the tuberculosis eradication program is strongly recommended. Continued research on the refinement
of tuberculosis testing, including minor species, is strongly recommended by this committee.

6. IMPORT—EXPORT—The committee commends USDA for its renewed efforts to prevent the entry of exotic diseases and pests into the United States. The quarantine stations must be continued with improved capability to intercept disease importations. Recovery of the costs of these programs should be supported by user fees adequate for recovery of expenses involved in an effective supervision of imports.

The committee believes USDA must increase its efforts to secure a larger share of the export trade for the U.S. livestock producer. Effort must be expended to convince importing nations that the United States has the expertise and facilities available to guarantee the validity of all claims made for the livestock exported. USDA must continue efforts to secure from importing nations fair and equitable import requirements based on their need to prevent disease import. The USDA is commended for its commitment to ensure all certification statements are valid.

7. VETERINARY BIOLOGICS—This committee urges that USDA continue toward legislative authority, through the proposed revision of the Virus Serum Toxin Act, and support and commend Veterinary Biologics on development of the concept, on an economic basis, whereby multiple laboratories may be utilized in production of licensed veterinary biologics.

8. NATIONAL VETERINARY SERVICES LABORATORIES—Veterinary Services Laboratories continues to be a critical link in the national network of animal diseases diagnostic and control facilities.

We urge that adequate construction and operational funding be provided to Veterinary Service Laboratories so that it may:

1. Give adequate support for field program diagnostic needs.
2. Provide reagents for state labs where not otherwise available.
3. Give comprehensive diagnostic reference support to state labs.
4. Provide training to state and/or industry diagnostic laboratory personnel.

9. COMPLIANCE—We commend the interstate inspection and compliance staff for their handling and concluding of increased cases.

Cases which concern veterinary accreditation are mostly minor and these should be handled administratively by the AVIC and state official.

Consideration should be given in accreditation cases to use of cash fines rather than suspension of accreditation.

Compliance officers should concentrate on repeat violators and on cases where the violation has resulted in disease spread.

We strongly support efforts to detect fraudulent practices in blood collection.

10. INTERSTATE REGULATIONS INFORMATION—We commend Veterinary Services for putting the state import regulations into the BIS
Further effort should be made to make this information directly available to practicing veterinarians.

We compliment the efforts of those responsible for identification for their efforts to improve quality of ear tags and back tags.

11. **PRV (AUJESZKY'S)**—The committee encourages APHIS to continue its work with the five pilot eradication projects. We, also, recommend the continued control of interstate movement of swine known to be infected with PRV. The work on development and testing of new vaccines should be continued. This committee commends the representatives of APHIS and the Swine Industry for their shared efforts to resolve this issue.

12. **SWINE HEALTH PROTECTION**—The committee recommends that better and more efficient ways be found to disclose those who are in violation of the regulation. The committee feels the number of inspections made during 1984 was sufficient, but wonders if it has reduced the number of feeders in non-compliance.

13. **F.S.I.S.**—The committee again strongly urges consideration for increased funding for T.A. Plants by USDA.

State inspection for these plants is accomplished at less cost. This can be done administratively and we would recommend funding at 90–10 ratio.

User fees for meat and poultry inspection should be carefully appraised before implementation. Thought should be given to inspection effectiveness and also to the manner state cost for these programs would be funded. User fees for federal cost and state funding for state costs would result in the downfall of state programs.

Increased effort from the TOP down in such things as blood collection and identification collection could be a great help to Animal Health Programs and we ask that this be done.

14. **CENTER FOR VETERINARY MEDICINE**—F.D.A.—The committee commends the center for Veterinary Medicine for its work in 1984. The approval of 34 major drugs or drug uses is a great improvement over what were being approved only a few years ago. This committee supports continued research necessary to determine the feasibility of using low level antibiotics in animal feeds, and supports regulations based on scientific facts. We encourage FDA to continue to examine the feasibility of simultaneous approval of animal drugs in all developed countries.

15. **TRANSPORTATION**—The State-Federal Relations Committee questions whether there is any present need for the expenditure of federal funds for the development of livestock trailers and railroad trucks. Efficiency in the transportation of live animals is a laudable goal, but at this time is a luxury which private industry can address with greater effectiveness.

16. **AGRICULTURAL RESEARCH SERVICE**—We commend ARS for their continued excellent research and in making great progress with the funds available. Many important research projects are in progress and we look forward to the results.
We support moving the Blue Tongue Research Laboratory. This facility should be placed where there is adequate safety for program and personnel with the least cost.

We strongly suggest that accelerated research be done on treatment and diagnosis for heartwater and that cooperation be extended to the Caribbean Islands to eradicate the ticks responsible.

We support continued research in regard to international movement of embryos and other genetic material.

We are very pleased with the continued communication between ARS and APHIS which greatly enhances our ability to control disease.

Responses to USAHA resolutions were quite satisfactory and encouraging.
The transmissible disease of swine committee convened at 1:30 p.m. on Wednesday, October 30, 1985. Eleven committee members and 45 guests were present.

The meeting was centered around the theme “The current and potential applications of biotechnology to swine health and production.” Dr. David Reed of Molecular Genetics, Inc., Minnetonka, MN, gave an overview of recent successes of biotechnological approach to the production of vaccines and diagnostics. He indicated that molecularly engineered vaccines against pseudorabies virus and transmissible gastroenteritis virus will probably be available within the next twelve months. In addition a subunit based diagnostic test to detect pseudorabies virus antibody will be released. Monoclonal antibody products will soon be available to treat neonatal E. coli diarrhea in pigs. Dr. Reed also gave an overview of the advantages and disadvantages of live vs killed molecularly engineered vaccine.

Dr. Erwin Workman of Agritech Systems, Inc., Portland, ME discussed recent advances in the development and use of rapid diagnostic tests. He addressed the development of new techniques which may be used for high volume testing in facilities such as diagnostic laboratories, and for low volume use at the practitioner or producer level. He emphasized technology is now in place to develop and produce these tests for a wide variety of assays if a large enough market volume can be established. Dipstick type tests are already on the market for pregnancy diagnosis, canine brucellosis, feline leukemia, toxoplasmosis, etc.

Dr. David Thawley, University of Missouri, gave a paper which discussed the impact of new practitioner based diagnostic tests on the animal industries. He noted that such tests will become increasingly available, and at least one for the diagnosis of a federally regulated disease will be available within the next twelve months. Decisions need to be made soon as to how test kits for regulated diseases will be controlled, so as to permit their most efficient use. He suggested one means of control where a reportable disease was involved would be to—
—regulate the sale of tests by control at the state level.
—report all results to state officials.
—account for all unused test kits.

The use of such tests will shift the responsibility of reporting notifiable disease outbreaks from the licensed diagnostic laboratories to the practitioner level. Such a shift could create a conflict of interest at the practitioner-client level. Dr. Thawley stressed that test kits for production limiting diseases could become most important tools for use by the health maintenance practitioner.

Dr. A. W. Smith, Oregon State University, presented an update on vesicular exanthema and related caliciviral diseases. He reported the identification of a new serotype SMSV #13 from marine mammals of the California and Oregon coasts. Serological evidence of infection with the serotype has been found in cattle from several west coast areas.

Experimentally the serotype is extremely virulent in swine and readily transmits horizontally. He proposed that an expanded research effort is needed to study the epidemiology and to develop new serologic tests which could be used for rapid diagnosis and surveillance. Since the gross lesions of VE are indistinguishable from other swine vesicular diseases, a rapid test for use on tissue specimens is needed. The committee passed a resolution which endorses continuing the ongoing cooperative calicivirus research program at the current or increased funding levels for the next three to five years and that additional funds be set aside to initiate a federal in-house calicivirus research program.

The meeting was adjourned at 4:15 p.m.
REPORT OF THE COMMITTEE ON WILDLIFE DISEASES

Chairman: E. Tom Thorne, Laramie, WY
Vice Chairman: Victor F. Nettles, Athens, GA

W. D. Bolton, VT; W. W. Buisch, MD; D. R. Cassidy, IA; A. H. Dardiri, NY; G. A. Erickson, IA; M. A. Essey, MD; J. B. Finley, TX; D. J. Gilhooley, HI; J. H. Gray, CO; F. A. Hayes, GA; D. A. Jessup, CA; D. C. Johnson, GA; W. E. Ketter, MD; R. J. Lee, VA; H. A. McDaniel, MD; J. W. McVicar, NY; E. V. Morse, IN; J. S. Smith, MD; R. K. Stroud, WI; C. D. Stumpff, KS; A. Thiermann, IA; G. S. Trevino, TX; and W. G. Winkler, GA.

The Committee on Wildlife Diseases met at 1:30 p.m., October 31, 1985. In the absence of Chairman Thorne, Vice-Chairman Nettles presided. The Committee’s Report for 1984 was reviewed and items which required additional work were discussed as Old Business. Summary statements with the Committee’s recommended action are cited as follows:

OLD BUSINESS

1. Compensation for Relocation of Wildlife in the Event of Depopulation as an Essential Measure for Preventing Spread of a Dangerous Contagious Disease

In the event of a foreign animal disease outbreak in the United States, the United States Department of Agriculture has the authority to reimburse farmers for livestock and poultry that must be destroyed in order to stop the spread of disease. The USDA also has the power to destroy wild animals; however, there are no provisions for restoring wild populations.

It can be predicted that killing wild animals will be an extremely unpopular activity which will evoke a strong public reaction against any associated disease control program. Therefore, all preparations should be made to make this unpleasant event as acceptable as possible, including provisions for wildlife restoration. Within the past year, communications on this subject have been shared among the USAHA, the International Association of Fish and Wildlife Agencies (IAFWA), and the Office of the Administrator, APHIS, USDA. All three parties are supportive to the concept of wildlife restoration provisions, nevertheless, it has become obvious that current authorities held by the USDA are not sufficient to allow such a use of USDA funds.

In the Fish and Wildlife Health Committee of IAFWA, a “white paper” is being prepared concerning past instances of wildlife depopulation and predicted future problems. There are plans for this “white paper” eventually to be used to approach members of Congress in hopes of legislative action. The International intends to have the “white paper” reviewed by the USAHA prior to any attempts at legislation.
**Recommended Action:**

The Wildlife Diseases Committee should proceed on this matter in cooperation with IAFWA. Copies of the “white paper” should be made available for comment to member agencies of the USAHA and to APHIS, USDA.

2. **Avian Influenza in Wild Waterfowl**

   Last year, Drs. Robert Webster and Victor Nettles reported to the Committee on wildlife surveillance during the lethal avian influenza outbreak in Pennsylvania and surrounding states in 1983–1984. Surveillance had demonstrated that wild birds were not disseminating lethal H5N2 avian influenza virus among poultry. After that report, Dr. Webster’s laboratory, in collaboration with the Southeastern Cooperative Wildlife Disease Study, The University of Georgia, continued to test wild waterfowl in the quarantined areas.

   A dramatic increase in the incidence of avian influenza viruses occurred in waterfowl (primarily ducks) during the summer and fall of 1984. One hundred seventy-one ducks were found infected with 13 different subtypes from June to November, 1984, none of which were considered pathogenic to poultry. This sudden increase in influenza viruses was without concurrent evidence of influenza in the dense domestic poultry population in the Pennsylvania area. From this experience the following conclusions could be made:

   a. A large variety of asymptomatic infections with avian influenza viruses can be found in free-flying aquatic birds and that incidence of infection is greatest in the summer and early fall.

   b. Free-flying birds do not appear to be disseminators or reservoirs for the lethal H5N2 strain that caused the recent disease outbreak in poultry.

   c. Proper hygienic measures and separation of domestic poultry from wild waterfowl can be effective in preventing avian influenza viruses from spilling over from wild birds.

   **Recommended Action:**

   None required.

**NEW BUSINESS**

1. **Duck Plague—Recent Experiences in Maryland**

   Dr. John Shook, Maryland State Veterinarian, reported to the Committee on recent activities in regard to Duck Plague (Duck Virus Enteritis) in his state. A collection of ducks owned by the Maryland Forest, Park and Wildlife Service became ill and were tentatively diagnosed on September 5, 1985, as having duck plague. Virus isolation was confirmed on September 23 and the ducks were destroyed on September 25. This incident was handled cooperatively between the Maryland Departments of Agriculture and Natural Resources and no legal quarantine or indemnity was involved.
A second suspected outbreak occurred in captive-bred ducks on a private shooting preserve on October 6 and necropsy at the State Diagnostic Laboratory revealed lesions suggestive of duck plague. With the encouragement of the Maryland Forest, Parks and Wildlife Service, the Maryland Department of Agriculture placed a quarantine on the premise while virus isolation procedures were in process. The quarantine was lifted on October 24 after virus isolation attempts were not successful.

Dr. Shook elaborated on the problems encountered during these events, many of which would be applicable to other states in the Nation. Questions of interest were as follows:

A. The status of ducks held on shooting preserves could be debatable. Are they wildlife or poultry?
B. Regardless of their status as wildlife or poultry, what action should be taken when duck plague is suspected?
   1. Should the State Department of Agriculture or the State Fish and Wildlife Agency have authority to quarantine such ducks?
   2. When should the quarantine begin, at necropsy or upon virus isolation?
   3. How much time is reasonable for viral diagnostic investigations?
   4. How should day-by-day laboratory progress reports be handled?
   5. If infected with duck plague, should vaccine be used or should the ducks be depopulated?
   6. If ducks are depopulated, who will pay indemnity?
   7. After ducks are depopulated, what constitutes adequate cleaning and disinfection?

Recommended Action:

The Committee recognized that situations nearly identical to the recent problem in Maryland will recur on a continual basis. Cross communications between the USAHA Committee on Wildlife Diseases, the IAFWA Fish and Wildlife Health Committee, and the U.S. Fish and Wildlife Service should be initiated to develop guidelines directed toward the above questions. Once developed and found mutually agreeable to all the aforementioned parties, the guidelines can be used by State Departments of Agriculture and/or Fish and Wildlife as problems arise.

2. Mycoplasmosis in Pen-reared Wild Turkeys

Restoration of the wild turkey in the United States has been one of the most noteworthy successes of the wildlife management profession. This species has rebounded from the brink of oblivion to a status of abundance, and wild turkeys are found today in areas beyond the historic range. Many turkey biologists consider the move away from the release
of game farm raised wild turkeys to the use of live-trapped native wild turkeys as a major turning point in turkey restoration. Although data on this subject are limited, it has been commonly thought that game farm wild turkeys may suffer high post-release mortality due to diseases, or worse still, introduce diseases into native wild turkeys.

In the past year, disease and parasite surveys have been conducted on pen-raised wild turkeys from 9 states through a cooperative project between the Southeastern Cooperative Wildlife Disease Study and the National Wild Turkey Federation. Although the project is not completed, it was noteworthy that clinical *Mycoplasma gallisepticum* was found in several animals in one subsample. Additionally, a diagnosis of *M. gallisepticum* infection was made in a released pen-raised wild turkey that was found in an adjacent state. These observations demonstrate that additional disease prevention measures are necessary in the commercial growing of captive wild turkeys.

Dr. I. L. Peterson of the National Poultry Improvement Program (NPIP) informed the Committee that game bird breeders can become members of the NPIP by conforming to that program and that the NPIP does include preventative measures for *M. gallisepticum*. Dr. Nettles felt that most state fish and wildlife agencies are philosophically opposed to release of pen-reared wild turkeys but often are unable to stop this practice. Generally, a permit from the state wildlife agency is required to buy, hold, ship, or release pen-raised wild turkeys, and it may be feasible for states to require that the turkeys be from NPIP flocks.

**Recommended Action:**

The Wildlife Diseases Committee recommends that State Fish and Wildlife Agencies be apprised by the USDA of game bird breeders currently enrolled in the NPIP. The USAHA should support State Fish and Wildlife Agencies in stipulating that pen-reared wild turkeys should meet NPIP standards.

### 3. Brucellosis in Wild Swine

The topic of brucellosis in wild swine had not previously been discussed in this Committee, however, a report was given to the Subcommittee on Swine Brucellosis last year at the USAHA meeting in Fort Worth, Texas. At that time, evidence of *Brucella suis* infection had been obtained in wild swine in 7 states. Since then, swine brucellosis also has been diagnosed in wild hogs in Texas.

Dr. Paul Doby, Chairman of the Swine Brucellosis Subcommittee asked for assistance in contacting fish and wildlife agencies to seek better control on the intra- and interstate shipment of wild swine. As a result, arrangements were made for Dr. Doby to attend the annual meeting of IAFWA and to speak at the General Business Session: Dr. Doby's comments were entered into the Proceedings of the IAFWA meeting. Furthermore, a memorandum was sent from the Inter-
national's Executive Vice President to all of its governmental agency members encouraging them to work with animal health authorities to: (1) stop indiscriminate relocations of wild swine of unknown disease status and (2) to minimize future contact of infected wild swine with domestic swine.

**Recommended Action:**

The Wildlife Diseases Committee and the Swine Brucellosis Subcommittee should continue to push for intra-agency cooperation between animal health and wildlife officials at the state level.

4. **Model Regulations for Control of Livestock and Poultry Diseases in Zoological Animals**

The Committee discussed the staggering volume of intra- and interstate movement of animals classified under the nebulous category of zoological animals. These movements are manifest most clearly at exotic animal auctions at which all kinds of zoological animals are bought and sold. There are 15–20 large scale auctions held annually in the United States. During a recent 5-day auction, approximately 4,000 animals were sold, including over 100 species of birds and 75 species of mammals. At least 5 species of endangered animals were offered for sale. Buyers and sellers from 40 states were represented as well as Canada, Australia, Africa, and England.

Numerous organizations and individuals are involved with the zoological animal trade including zoos, rehabilitation centers, animal breeders, circuses, wholesale/retail dealers, pet owners, hunting preserves, petting and roadside zoos, and fur raisers. Auctions represent the overt sale and traffic of zoological animals; however, substantial movement also is likely through private treaty. The disease implications of this activity should not be taken lightly. Consideration for livestock and poultry diseases such as brucellosis, tuberculosis, malignant catarrhal fever, avian influenza, exotic Newcastle disease, etc., must be made. At present it is apparent that disease prevention measures practiced at zoological animal sales are minimal or inadequate. In addition to disease considerations, the current status of zoological animal trade has prompted concern from animal welfare groups and conservationists. The release or escape of exotic animals into the wild has become a major worry to state fish and wildlife agencies.

**Recommended Action:**

The Wildlife Diseases Committee recommends that a Model Regulation be developed for state governments to use in the control of livestock and poultry diseases that could be harbored or spread by zoological animals. The Committee prepared a resolution that would ask APHIS, USDA, to take the lead in developing this Model Regulation.

5. **Leptospirosis Survey in Wildlife**

Dr. Thiermann reported on serological prevalence studies for lept-
spirosis in wild swine and white-tailed deer. Serum samples were collected from southeastern and southwestern states and Hawaii. Among wild swine, prevalences varied from 9 to 53.8%. Most reactions were to serovar *bratislava*. This serovar was unknown for the United States until recently when it was isolated from Iowa swine at slaughter.

Most deer populations studied showed no serologic reactions; however, 4 of 5 (80%) on Lacassine National Wildlife Refuge (NWR) and 2 of 6 (33%) on Tensas River NWR were seropositive to *pomona* in Louisiana. Reactors to *grippotyphosa* were detected in Alligator River and Delta NWRs in North Carolina. Serovar *pomona* is uncommon in deer, and serologic evidence could indicate possible exposure from other wildlife hosts or domestic animals. Although serologic studies are good indicators of prevalence, Dr. Thiermann stressed that the isolation of leptospires is essential for a better understanding of the disease in a certain population. Isolation attempts conducted during this study did not yield leptospires.

Dr. Thiermann also reported on ongoing studies on leptospirosis in sea lions. A serologic survey among Alaska sea lions this year yielded no serologic reactors. However, samples submitted by Drs. Dierauf and Vedros in California showed high titers to serovar *pomona* among all samples submitted. Isolations were made from kidneys during a 1980 and a 1984 outbreak. The organisms belong to serogroup Pomona and are currently being further characterized.

**Recommended Action:**

None required.
REPORT OF THE COMMITTEE ON ZOOLOGICAL ANIMALS

Chairman: M. S. Silberman, Atlanta, GA
Vice Chairman: R. L. Crawford, Hyattsville, MD

R. A. Bowen, CO; Lanny H. Cornell, CA; P. M. Eppele, MD; Gene Erickson, IA; J. A. Farrar, FL; Milton Friend, WI; Allan Furr, MD; E. E. Grass, CA; D. E. Herrick, MD; Werner Heuschele, CA; Calvin Lum, HI; C. J. Mikel, OK; G. W. Patterson, TX; Janet B. Payeur, TX; G. P. Pierson, MD; Jeanne Roush, DC; K. C. Sherman, MO; E. Tom Thorne, WY; R. J. Yedloutsching, NY.

The meeting was called to order at 1:30 p.m. by Morton Silberman, Chairman. This meeting was attended by 12 committee members and 16 guests. An agenda handed out at the meeting was followed.

Dr. Gary Colgrove, NVSL presented a summary of 9 years data on Mycobacteria isolated from exotic animals and wildlife. *Mycobacterium avium* was isolated from 47.7% of the cases, *M. bovis* from 20.6% and *M. tuberculosis* from 12.9%. These three accounted for 81.2% of the *Mycobacterium* isolated. The isolates reported were from 23 states and territories, some 18 mammal species, as well as 250 birds.

Dr. Edward DuBovi, Cornell University reported on a virus isolated from llama and alpaca that destroys the retina and optic nerve eventually leading to blindness. The virus was also isolated from two zebra that showed the disease and may be found in fallow deer. A request was made for llama and alpaca sera to screen for virus. The virus has been identified as Equine Herpes Virus I.

Dr. Werner Heuschele, San Diego Zoological Society, presented updated data on MCF. He has evaluated over 4800 sera and is convinced that the wildebeest presents the greatest potential danger for transmission. The virus neutralization test is the most specific diagnostic tool available. Dr. Heuschele offered a resolution related to the serum-virus neutralization testing for MCF. This resolution passed without dissent.

Dr. Heuschele also reported on rinderpest, stating that there is no reason to believe this virus ever occurs in the carrier state. Animals that have been infected with rinderpest virus either die or develop a life long immunity. He, as well as other members of the zoological community, questioned the need to screen for this agent when importing wild ruminants, especially, with the critical condition some of the endangered species are finding themselves in.

Bluetongue was also addressed by Dr. Heuschele and he posed the question of whether or not animals infected with this agent are not immune for life. The need for continuous research with this virus was agreed to by the entire committee.

Dr. Ed Ramsey, Oklahoma City Zoo, presented a policy adopted by the American Association of Zoo Veterinarians for the testing and shipment of non-domestic ruminants with regard to Malignant Catarrhal Fever. The
previously mentioned resolution addressed these concerns, and as stated was adopted without dissent.

Dr. Milton Friend, USDA, updated the committee on the Inclusion Body Virus of Cranes. Little is presently known about the agent, but it is potentially a problem and more sera is needed for evaluation. It is very apparent that cooperation is needed between USDA and USDI for testing and quarantining for this disease. The committee strongly recommends that this be resolved at the administrative level by both agencies.

Dr. Richard Crawford, USDA updated the committee on some proposed changes in Title 9, CFR, parts 1, 2, and 3. He also made copies of the new federal health certificate for the interstate and international shipment of dogs, cats, and non-human primates. Dr. Silberman reminded the committee that research activities are becoming more prevalent in zoological parks and that a concern for regulating these activities has been expressed by various humane groups.

Dr. Allen Furr, USDA, told the committee of proposed change in the regulations for importing elephants and rhinoceros species. These animals would have to be inspected and treated for ectoparasites. As a result of discussions on the disposition of United States origin animals in post entry quarantine zoos, a resolution was offered and passed without dissent that would ease this situation.

Dr. Silberman discussed the problem states find themselves in when trying to regulate the interstate movement of non-domestic species. A resolution requesting APHIS to develop model legislation that the various states could adopt was introduced by Dr. Heuschele and passed without dissent.

The meeting was adjourned at 4:30 p.m.
THE CURRENT STATUS OF NADDS
Phyllis York, DVM Veterinary Services, APHIS, USDA,
Hyattsville, Maryland
Oct. 31 1985

To acquaint you with the status of the National Animal Disease Detection System (NADDS), we present information about three areas: (1) current activities of NADDS and the cooperators; (2) the future plans for states and projects that assist the development of NADDS, and (3) the growing pains we have encountered and how we plan to convert these growing pains into program developments.

Introductory remarks about NADDS include a reaffirmation of its goals; that of acquiring statistically valid information on the incidence, prevalence, trends, and economic impact of livestock and poultry diseases, both infectious and non-infectious. NADDS was planned as an interdependent, collaborative effort. Fortunately for the program, we have encountered dedicated and strong support from a variety of sources and colleagues. These associates have and will continue to contribute greatly to the program. Some of these contributions will be important parts of this session.

Current activities include the participation of six states: Ohio, Colorado, Tennessee, Iowa, California, and Georgia. The State, Federal, and often university veterinarians, through monthly interviews, are collecting information on various species.

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<th>Participating State</th>
<th>Cattle</th>
<th>Cow/Calf</th>
<th>Feedlot</th>
<th>Dairy</th>
<th>Hogs</th>
<th>Sheep</th>
<th>Poultry</th>
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<td>Michigan              +</td>
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The number of herds, species of interest, and starting dates vary from state to state.

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<thead>
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<th>Participating State and Number of Herds</th>
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<tr>
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<td>7/85</td>
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### PLANNED OR REINITIATED

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The implementation of this central activity, *data collection*, prompts methods development in supporting satellite areas: sampling of the population at risk, comparison and combination of list and area sampling frames, computerization and analysis of data, sub-sampling for diagnostic verification, and correlation and validation of information collected.

![Diagram of satellite developmental activities](chart.png)

A summary of satellite developmental activities shows a diversity of projects, the responsibility for which is assumed jointly by universities, States and APHIS.

**Cooperators**

- Ohio State University
- University of California
- State of California

**Projects in Process**

- Veterinary Micro-Economics
- Validity of Data
- Sampling Frames
- Data Management
- Data Validation
- Lab Diagnostic Tests, for Mass Screening
- Methods Development for Data Collection for Large Dairies
- Determination of Frequency of Data Collection
The current status of NADDS

Iowa State University  
State of Iowa  
- Data Management and Analysis  
- Sub-sampling Hogs Using Diagnostic Tests  
- Serum Bank  
- Teaching Methods  

Colorado State University  
- Data Management  
- Sub-sampling Feedlot Diagnostic Tests  

University of Georgia  
Extension Service  
- Poultry Survey Forms  
- Sub-sampling Poultry  

Cooperators  
Cornell University  

Johns Hopkins University  

Michigan State University  
State of Michigan  
- Data Management  
- Sub-sampling—Environmental Factors and Noninfectious Diseases  

Centers for Disease Control  
U.S. Public Health Service  
Atlanta, Georgia  
- Data Management  
- Sub-sampling—Environmental Factors and Noninfectious Diseases

Additional Projects Planned  
Cornell University  
- Adaptation SNOVET to NADDS requirements  
- Computer Program Development  

Johns Hopkins University  
- Survey/Design  
- Data Management  
- Statistical Analysis  

Michigan State University  
State of Michigan  
- Data Management  
- Sub-sampling—Environmental Factors and Noninfectious Diseases

Future plans for NADDS, in addition to the proposed joint activities with States and universities, include subjecting our methods and first results to a panel of experts from outside APHIS for systematic evaluation. This panel will offer constructive criticism and direction to NADDS.

Expansion of NADDS to additional States will occur within carefully defined limits. The States to be added will strengthen the statistical validity of the results, have food animal populations that are underrepresented, or be contiguous with States already participating in NADDS. Michigan, Wisconsin, and Kansas have begun the initial steps toward participation. A number of other States have expressed interest in beginning the program.

Perplexities arise as a result of growth increment from a pilot project to a national system. These difficult-to-define stages on a continuum are often called transitional stages. NADDS is in transition. NADDS is past the pilot trial stage but still relies heavily on further methods development and explorations of procedures and process to accomplish its goal. This exploration gives flexibility to the program, especially as it is implemented to address interests of different States; however, it is evident that constancy and consistency describe basic elements of a national system. It
is foreseen that although flexibility in the process and State-by-State differences will be continual, there will be a need for a core of information, uniformly reported that will be suitable for national purposes.

One of the more significant growing pains of NADDS is the management of data. For rapid retrieval of the computerized data, a coding system must be usable. Because of our difficulty in adapting SNOVET for NADDS purposes, a cooperative agreement is planned to solve this operational problem. Part of the cure for the growing pains of NADDS is the consolidation and examination of data we have available now, coordinating this with the various ongoing activities to then again, add States to the system, in quest of our goal.

Hinging on efficient data retrieval is the timely return of information to the producer. Our experience thus far shows this to be an essential element of the program. The need for valid data and results while developing the methods to obtain it characterizes this dilemma.

The realization that veterinary preventive medicine, surveillance being a component, demands a modified set of skills different from usual regulatory tasks has made adequate training for NADDS perplexing. We are relying on the constructive evaluation of the interviewing veterinary medical officers for changes in instruction.

Confidentiality has been a component of NADDS difficult by those responsible for information about reportable disease to accept. Although not an operational problem, this requirement is a source of reticence for some.

To preserve the autonomy and leadership of the States, while working toward a cohesive, national system presents a challenge to all participants. So far, the cooperative nature of the individuals related to NADDS has turned this challenge into program productivity. The excitement of working on NADDS has not waned, rather with the first look at the results NADDS will be able to give us, the enthusiasm on our part has increased. Acronyms describe complex entities. With one word, NADDS, this program might also stand for Network, Achievement, Dynamic, and Significant.
STATUS OF THE INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE'S PROPOSAL FOR AN INTER-AMERICAN SURVEILLANCE AND REPORTING SYSTEM ON ANIMAL HEALTH

Dr. Héctor Campos
Director Animal Health Program
Inter-American Institute for Cooperation on Agriculture — IICA —
General Directorate
Washington Office
Washington, DC

Background Information

At the end of 1984 IICA requested the collaboration of Dr. Frank J. Mulhern in designing a reporting system on animal and plant diseases and pests that could be used at the hemispheric level.

With the cooperation and support of USDA/APHIS Dr. Mulhern prepared a proposal for such a system, which was sent to 27 member countries of IICA jointly with a questionnaire in order to assess the need for an information system at the hemispheric level, the interest of the governments in participating, as well as the existing capacity for setting up a reporting system.

IICA also sought to determine the agencies or groups which could provide information as well as the opinion of the authorities on the advantages and disadvantages of a reporting system at the hemispheric level.

The preliminary results of that survey were analyzed by Dr. Jorge Vargas and presented on behalf of IICA before the Second Meeting of the Inter-American Commission on Animal Health held in Brasilia, D.F., on May 1985. Further data received from other countries permitted a better knowledge of the interest of the surveyed countries.

Results of the Survey

The survey showed a positive response to the development of an Inter-American Reporting System in the field of animal health as well as in the plant protection field.

On this occasion, we will only discuss the results obtained related to the animal health field.

From a total of 27 countries from Latin America and the Caribbean surveyed, 18 of them responded to the questionnaires, which represents a 66.6% response.

In tables 1, 2, 3 and 4 the main results of the survey are presented, the countries having been divided in four groups according to the four areas of geographic distribution of IICA.

In accordance with the obtained results, 88.8% of the countries are developing an information system within the country or at least collecting
and processing data on animal health. All countries report to various international systems developed by OIE, FAO, PAHO, IICA, JUNAC and OIRSA.

94.4% of the countries have personnel collecting data, even when that personnel is accomplishing other various duties.

Only 33.3% have computers available; nevertheless the rest of those surveyed countries responded that they could obtain this equipment.

94.4% of the countries have Laboratory diagnosis capability, in many cases with limitations to certain tests and procedures.

All countries expressed interest in the development of an inter-American reporting system through IICA and 94.4% indicated interest in participating in such a system. 88.8% of the countries would be interested in starting a pilot program.

In relation to the commitments of support for the proposed system, all countries could provide information on exotic diseases and pests as well as data on endemic diseases and pests of economic importance. Only 38.8% of the surveyed countries could provide specific personnel for the system, and only 50% could commit vehicles for that specific purpose.

**Current reporting systems in use in the Americas**

There are various systems in Latin America and the Caribbean developed by different international, regional and sub-regional organizations.

a) The International Office of Epizootics —OIE— has 14 member countries in the Americas and from those countries regularly receives information in accordance with Lists A and B of that organization. IICA is obtaining information for OIE from the non-members countries of that bureau. OIE publishes monthly and annual reports as well as communications on emergency situations and outbreaks.

b) The Food and Agriculture Organization of the United Nations —FAO— collects information on animal health from most of the countries of the Americas, and jointly with OIE and World Health Organization —WHO— publishes the Animal Health Yearbook.

c) The Pan American Health Organization —PAHO— through the Pan American Foot and Mouth Disease Center —PANAFTOSA— develops an Epidemiological Surveillance System on Vesicular Diseases with weekly and monthly reports. This system covers Central and South American countries.

d) PAHO is also developing a surveillance and information system on animal health in Central America and Panama that covers different diseases besides vesicular diseases.

e) Through the Pan American Zoonosis Center —CEPANZO—, PAHO collects information on incidence of rabies and equine encephalitis in American countries.

f) IICA is developing an Animal Disease Information System for the
Caribbean Area which covers different diseases and publishes quarterly reports.

g) OIRSA, the subregional organization on animal health and plant protection for Mexico, Central America and Panama, and JUNAC, the sub-regional organization for Andean countries, also collect information on animal health but mainly for the development of their projects for prevention of exotic animal diseases.

**Design of the IICA proposal**

The purpose of the IICA proposal for an Inter-American Surveillance and Reporting System on Animal Health is to unify all the different systems currently in use for various organizations in different countries of the American hemisphere, in order to standardize procedures for collection, analysis and processing of data as well as for the publication and distribution of the information.

The products of the system will be:

a) Emergency reports on animal exotic disease outbreaks of List “A” of OIE in countries previously free of those diseases.

b) Monthly and annual reports on priority diseases from lists “A” and “B” of OIE.

c) Reports on morbidity and mortality, and estimates of economic impact.

In chart No. 1 is shown the information flow for the proposed system and in chart No. 2 is shown the scheme of the system.

The proposed system will have two levels:

a) The in-country level, in which the system will be developed according to special characteristics, infrastructure and resources of each country, trying to follow standardized procedures.

b) The international level, with the participation of all international, regional and subregional agencies acting in animal health in the Americas under a unified reporting system.

**Future steps to be taken**

During the meeting of COINSA held in Brasilia, D.F., on May 1985, the Delegates requested that IICA promote a meeting with those responsible for information systems in the interested countries, and with the representatives of the international organizations, to study the mechanisms for the establishment of a unified information system on animal health at the hemispheric level, taking into account the systems in use through OIE, PAHO and other agencies.

IICA will call for that meeting during the first quarter of 1986. In the meantime, contacts with the 18 interested countries are being established in advance to define their participation in the system.
<table>
<thead>
<tr>
<th>COUNTRIES</th>
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*Positive answers.*
## Status of the Inter-American Institute

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### Results: *

|                  | **94.4%** | **33.3%** | **66.7%** | **94.4%** |

*Positive answers.*
### TABLE 3

**INTEREST IN THE PROPOSED SYSTEM**

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<thead>
<tr>
<th>COUNTRIES</th>
<th>DEVELOPMENT OF SYSTEM BY IICA</th>
<th>INTEREST OF PARTICIPATION</th>
<th>INTEREST PILOT PROGRAM PARTICIPATION</th>
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*Positive answers.*
### SUPPORT COMMITMENTS FOR THE PROPOSED SYSTEM

<table>
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<tr>
<th>COUNTRIES</th>
<th>DATA ON EXOTIC DISEASES &amp; PESTS</th>
<th>DATA ON ENDEMIC DISEASES AND PESTS ECONOMIC IMPORTANCE</th>
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*Positive answers.
INFORMATION FLOW

ANIMAL HEALTH SERVICES IN EACH COUNTRY

IICA or PAHO OFFICE IN EACH COUNTRY

PROCESSING CENTER UNIT AT HEMISPHERIC LEVEL (TO BE DETERMINED)

INTERNATIONAL AGENCIES

PUBLICATION OF RESULTS
CHART No. 2
SCHEME OF THE SYSTEM

COMPONENTS

I INFORMATION AND ALERT

II MORBIDITY AND MORTALITY

SOURCES OF DATA

- Veterinarians
- Diagnostic Laboratories
- Slaughter houses
- Clinics and hospitals
- Universities
- Research institutes

PROCESSES

- Producers
- Veterinarians
- Statistical sampling
- Impact assessment
- Losses and costs

RESULTS FEEDBACK

- Emergency reports of exotic diseases outbreaks of List "A" of OIE in countries previously free of those diseases
- Monthly and annual reports of priority diseases from List "A" and "B" of OIE
- Reports on morbidity and mortality, and estimates on economic impact

PRODUCTS
Background

The Mexican Animal Health Program has been in search of information that provides reliable data for decision making during the implementation of programs related to control and/or eradication of enzootic and exotic diseases that threaten the country. The most significant attempts to obtain reliable animal health information are outlined below:

In 1954, after the outbreak of Foot and Mouth Disease (1946–1954), the Department of Agriculture and Livestock established a mutual agreement with the U.S.A., in order to set up a Commission for the prevention of Foot and Mouth Disease. This program was aimed at educating livestock owners and veterinarians throughout Mexico, so that they would report cases of vesicular diseases so as to avoid the reappearance of Foot and Mouth Disease. During 31 years of the Commission's existence, an effective surveillance system of vesicular diseases has been maintained. Thanks to this program, the county has been maintained free of Foot and Mouth Disease.

In 1968, in response to petitions made by livestock owners and veterinarians, the Department of Agriculture and Livestock began the creation of the National Network of Veterinary Laboratories. This Network is considered the key to the country's zoosanitary infrastructure and is actually comprised of 105 units, which have been built in the areas of major livestock activity. These laboratories have provided diagnostic support to livestock owners and veterinarians who in turn have permitted the taking of preventive and therapeutic measures for enzootic and epizootic diseases. The information produced has been used as the basis for constructing animal health programs and disease control measures.

In 1981, when confronted with the threat of the introduction of African Swine Fever and as a backup for the Hog Cholera control program, a pilot surveillance program specific for ASF and HC was initiated in which outbreaks were mapped geographically according to the earth's parallels and meridians. The Mexican Territory was divided into 2,600 quadrants. Each quadrant has an approximate surface of 960 sq. kms. In this way, complete information was obtained about the different characteristics of each area, of which the most important being: the location of production units, means of communication, natural barriers such as mountains, rivers, lakes, etc. This system has proved to be effective in the localization, follow up and control of epizootic outbreaks of hog cholera.

In 1982, this system was extended in order to cover 17 other “reportable” diseases.
Reportable diseases that are included in the disease surveillance system are:

**Bovines:** Rabies, Infectious bovine rhinotracheitis, Vesicular Stomatitis, Anthrax, Ticks (*Boophilus spp*) in free areas.

**Swine:** Aujeszky’s disease, Hog Cholera, Erysipelas, Transmissible Gastroenteritis (TGE).

**Poultry:** Salmonellosis, Newcastle disease.

**Equines:** Infectious anemia, Venezuelan encephalomyelitis, Influenza.

**Sheep and Goats:** Contagious ecthyma.

**Dogs:** Rabies, canine parvoviral gastroenteritis.

**Bees:** Acariosis.

From 1982 to 1984, we analyzed the advantages of the Minnesota Food Animal Disease Reporting System (MFADRS) and the development of the pilot plans in five states of the U.S.A. The formation of your National Animal Disease Surveillance (NADS) was evaluated as a system that could generate reliable information for enzootic diseases and also for some epizootic diseases. Those that do not fall into the category of the obligatory report disease or that lack an established program, but may cause grave economic production losses. Such is the case of Mastitis of differing causes, the pneumonias in their different forms, external and internal parasites and also animal death losses from different causes.

The advantages of this information system were presented to the Interamerican Institute for Agronomic Cooperation OEA (IICA), as a project for the development of an Interamerican System for Epizootiological Information and Surveillance (SIIVE). In July 1985 in the 2nd Reunion of the Interamerican Animal Health Commission (COINSA), the SIIVE was announced to the representative of IICA member countries as a model of surveillance system for this continent and the Caribbean area.

### Field Work

We began with the livestock region known as “Region Lagunera” or “La Comarca Lagunera” which is found in the state of Coahuila and Durango, and cover the counties of Gomez Palacio, Lerdo, Mapimi and Tlahualilo in the state of Durango—plus Francisco I. Madero, Matamoros; San Pedro de las Colinas, Torreon and Viesca in the state of Coahuila. The Nazas River irrigates an extensive region that is dedicated to the production of alfalfa, cotton and grapes. The climate can be described as semidesert and with extreme summer temperatures that read 42°C in the shade and in winter a minimum of −10°C.

The livestock population is made up of dairy herds that generally use good herd management techniques. The development of the poultry industry has been initiated as well as an increasing activity in swine raising, with installations that include environmental control.
The milk "shed" covers 211 premises with a total population of 71,872 milk cows. This population was used as the base for the pilot program as first step.

Field Plan

The regional headquarters of this "La Laguna" region livestock program, has placed special interest in the obtaining of reliable information from the livestock production areas with special emphasis on animal diseases and their economic impact. The goal of our program is to generate reliable information about production costs, sale and the identification of health problems that confront the livestock industry. Once these variables have been identified, with the information provided by the producers, the government will initiate technical aid programs in order to increment the livestock production in the Laguna region. The economic analysis of livestock production will be a basis that the government and private sector can use in solving problems that the producers face. Economic losses caused by animal diseases can then be better evaluated.

Statistical Design

The pilot program is based on statistical methods for selection of a representative sample of production units at a regional level. The methodology of selection of a representative sample was described in the SIIVE document. The population is stratification is carried out according to species, zootechnical function and other quantifiable variables in the first stage of milk cattle selection.

Of the 211 milk production units in the region with a total population of 71,872 milk cows, 8 strata were defined according to the population size. From each strata a random representative sample was taken which totalled 80 production units which are providing information. In Graph 1 the stratification of the "La Laguna" milk production units is shown.

<table>
<thead>
<tr>
<th>Strata</th>
<th>No. of Production Units</th>
<th>Selected Production Units</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1-100</td>
<td>62</td>
<td>23</td>
<td>29.3</td>
</tr>
<tr>
<td>2. 101-200</td>
<td>38</td>
<td>15</td>
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<tr>
<td>3. 201-300</td>
<td>28</td>
<td>10</td>
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<td>6. 501-600</td>
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<td>5.6</td>
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<tr>
<td>TOTAL</td>
<td>211</td>
<td>80</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Field Work

Questionnaires were designed that included a series of questions of statistical and economic nature, as well as those referring to animal health in order to evaluate the production costs, sales, animal health and the economic impact of animal disease. In order to carry out the surveys of the producers, 10 veterinarians with certain qualities were selected. These qualities were: a) Ability and willingness to convince, b) Work responsibility and interest in the information system, and c) Familiarity with the producers.

The system coordination on a local level is carried out by veterinarians who have been trained in the organization of information and who can call upon a group to advise them in the areas of economics, statistics and animal health. Together, the veterinarians and their group of advisers analyze the information and propose alternative solutions to the problem for which this system was created.

Data capture has been achieved using trained technicians. The participation of producers allows for the identification of problems in the level of production as well as animal health problems in the herd.

Results and Decision

The first survey has allowed us to categorize production units into three categories:

a) highly technified (highly mechanized)

b) technified (mechanized)

c) untechnified (unmechanized)

The characteristics of the cattle production population:

a) lactating cows

b) dry cows

c) calves

d) bulls

The type of feed used:

a) green chop

b) dry fodder

c) concentrates

d) harvests

e) minerals

The main problems in animal health during the month as well as other pathological conditions.

The number of animals culled by categories and main causes for this:

a) cows

b) calves

c) bulls
The total number of hectares or acres that are dedicated to the production of food. The milk production by Unit. The optimum capacity of the installations and the capacity that is actually used. The second survey allowed us to find out more about:

a) production costs.
b) number of culled animals and causes.
c) preventive measures that are being used in their cost.
d) the use of artificial insemination or natural service and its costs.
e) the number of births as well as the number of deaths and their causes.
f) the number of abortions.
g) the number of animals with reproductive problems and 
h) the presence of diseases and their costs.

Data is being analyzed by our livestock program headquarters in the “Comarca Lagunera.” Under assessment are agricultural economics, animal health programs, and incentives for increasing livestock production. Data processing has been carried out by our computer which has permitted short term results. The results of the information system will be made on the monthly, quarterly, and annual reports that give feedback to the producers, veterinarians, and government programs.
DISEASE SURVEILLANCE IN FOOD-PRODUCING
ANIMALS IN CANADA

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Disease surveillance in Canada is dependent upon the activities of three principal veterinary groups. Private practitioners, as the most intrinsic link to the livestock industry, are relied upon by both Provincial and Federal Veterinary Services to bring to the latter's attention significant situations which fall within the jurisdiction of these respective government networks. Such reporting, however, except where mandatory under Federal or Provincial law, depends upon a myriad of factors varying from practitioner interest to proximity of government offices, and as such is not adequate for the ongoing surveillance of the livestock community. For that very reason, it is almost a decade since Agriculture Canada discontinued a voluntary reporting system based upon practitioners.

Provincial veterinary services offer field extension services for the investigation of specific 'program' diseases of particular interest to the province involved. However, their continued major emphasis is the provision of diagnostic services for practitioners and livestock owners. Their daily submissions reflect on factors as basic as owner willingness to pay where applicable and veterinary interest in or inexperience with disease conditions they encounter. Their surveillance, therefore, is essentially passive, although it covers a broad range of disease conditions, and is not representative.

The Federal service, in contrast, while emphasizing active surveillance, limits itself to the number of diseases it surveys. Therefore, while it provides an excellent, active surveillance for bovine brucellosis and tuberculosis, it is extremely limited in its awareness of trends in other common conditions such as Infectious Bovine Rhinotracheitis and Leptospriosis.

It was in recognition of the relative weakness of the existing surveillance that Agriculture Canada sponsored a 1981 symposium on the subject. At that meeting of Industry, Practitioners, Universities and Governments two theories emerged. The Federal service proposed the adoption of the NADDS approach. The University group with some Provincial support suggested that the Federal government support the development of systems at the practitioner and client level, from which information could later be syphoned and accumulated for national reporting. The only point of unanimity centred on the leadership role, all parties requesting that it be left to the Federal service.

During the intervening years, Federal representatives liaised closely with NADDS developments and investigated the progressive approaches
of other nations. In 1984 and 1985 a Federal working group reviewed the entire surveillance concept and the relationship between the divergent opinions of 1981. They proposed a NADDS type approach as part of a surveillance system that would also seek to enhance existing passive systems through more active sharing of data. However, it was proposed that the active surveillance record production as well as disease values, as an extension of the NADDS approach.

In 1985, the working group met with a sub-committee from the 1981 symposium, and discovered that opinions were much closer than in 1981. In fact, in the interim, not only had the Federal department begun to support the development of microcomputer applications on farms, but at least one Provincial service had begun active surveillance at auctions. During a discussion on shared activity, memos of understanding and provincial programs it was agreed that within the Federal service there was a place for a NADDS approach that incorporated the assessment of both disease and production parameters on randomly selected farms, and that consultation with Provincial governments would best guarantee its success.

On the basis of that understanding, the working group is preparing a position for Senior Management, toward consultation with production and veterinary experts in the preparation of a surveillance outline for an initial species, perhaps swine.
THE USE OF NADS AS A TEACHING AID IN COLLEGES OF VETERINARY MEDICINE

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Veterinary Clinical Sciences, Iowa State University

The National Animal Disease Surveillance (NADS) program is serving as an important element of material for teaching courses in epidemiology, disease surveillance and production management at the Iowa State Veterinary College. Lectures in the above areas have been minimal to nonexistent in the veterinary curriculum. This information is considered to be invaluable for finding purposes for the administration of our college, to make adequate planning to better serve the livestock health needs of the state and region. The administration is elated at long last to have factual information for these purposes.

One year of data of disease surveillance from 60 farms selected (by random walk) in Iowa by NADS statisticians, indicates economic problems in 30-40% of the producers because of disease management which is coupled with the current depressed farm economy.

The Veterinary College has the responsibility of collecting data from four farms of the 60 selected. Students from the epidemiology and preventive medicine rotation accompany the clinician on monthly farm visits for recording data. This has been an excellent opportunity for students to visit the farm facilities and have an in depth look at the producer’s problems. The interaction of the students and producers to date has been educational for both parties and the producers look forward to the next visit.

The material accumulated from all 60 producers is also used as teaching material in the epidemiology section of the preventive medicine course to the second year veterinary students. The poor results on some farms which use apparently optimal levels of antibiotics and vaccines is emphasized. Current factual information is appreciated by the students.

Fourth year veterinary students and graduate students are now being offered an elective course in clinical epidemiology and production medicine. Basic epidemiology and production medicine material introduce the course. The students are then given one years data from each of the swine production units from the NADS program. This includes the swine inventory, facilities, disease prevention expenditures, cost of disease treatment and losses, and serology. The students are instructed as to entering data on a computer disc and are required to make a presentation as to the producers problem areas and methods of improving the producers operations. The course has been received very well and is over subscribed.

A grant from the Iowa Technology Council to our section has added increased emphasis to disease surveillance in Iowa. This money has also allowed more time for our staff to analyze and present data. We are very close to being designated a separate section. Our office and laboratory are
frequently visited by veterinary students, graduate students, faculty, researchers, practitioners, and regulatory people for information.

I would also like to emphasize the importance of cooperation of many people and departments for this type of program to be functional. The Diagnostic Laboratory, State Veterinarians (State and Federal) and many sections and individuals at the Veterinary College are needed and all have been very cooperative in this venture.
USING THE UNITED STATES DEPARTMENT OF AGRICULTURE NATIONAL ANIMAL DISEASE SURVEILLANCE SYSTEM TO MONITOR HUMAN HEALTH HAZARDS

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The concerns of the American public about toxic chemicals in the environment are rapidly increasing. People are demanding to know if they have been exposed to hazardous chemicals in their residential environments, and if so, what these exposures mean in terms of their future health.1 In response to these public concerns, researchers are looking for highly sensitive and specific ways for detecting human exposures to environmental contamination and the health effects that such exposures may cause. For several reasons, much of this needed methodology could be provided by adding surveillance of animal chemical exposures to the existing National Animal Disease Surveillance System (NADS) of the United States Department of Agriculture (USDA).

The concept of using animals systematically to provide early warnings of human health hazards due to chemical exposures dates back to when canaries were first used in coal mines.2 Since canaries are more sensitive to the effects of gas exposures than people, they were excellent sentinels of rising gas levels in the mines. Taking their cues from the canaries, coal miners could tell, early on, when they were likely to be exposed to dangerously high levels of gas. Accordingly, they could take preventive measures to limit their exposures. Simply stated, they could exit to safety in time.

Domestic livestock in the United States today share many of the characteristics that made canaries sensitive indicators of environmental hazards in the coal mines. First, like canaries, domestic livestock live with people in an environment that is likely to be contaminated. As an indicator of how widespread the problem of contamination is in our country, the General Accounting Office of the U.S. Government estimates that the number of waste sites in the U.S.A. in need of corrective action may be in excess of 378,000.1

Second, like the coal-mine canaries, domestic livestock are likely to be exposed to high levels of chemicals before people who live in the same areas are exposed. This sensitivity results from the behavioral characteristics unique to livestock. Pastured swine and cattle live in the outdoor environment. Therefore, the time during which they may be exposed to contaminants in that environment is necessarily greater than that for humans. Foraging swine and grazing cattle may ingest substantial amounts of soil with the plant materials that they consume. Soil intake may average as much as 7%-8% of the total amount of dry matter ingested.3,4
This amount far exceeds the amount of soil or dust that people living in the same environment would typically ingest or inhale.\(^5\)

Thus, the potential exposures of cattle and swine to soil contaminants are proportionately greater. Similarly, the potential exposures of cattle to water contaminants are also amplified. The daily water consumption of a lactating dairy cow may be as much as 45 gallons.\(^6\) For a 130-pound woman to match this potential exposure to water contaminants on the basis of body weight, she would have to drink 3.3 gallons of water a day.

A third characteristic that domestic livestock share with the coal mine canaries is that they exhibit adverse health effects much earlier than people who experience comparable exposures. This sensitivity is due to the shorter life spans of domestic livestock. Because of the rapid aging process, animal populations are likely to exhibit higher incidence rates for environmentally induced adverse health effects, such as reproductive problems, cancers, and immune system defects. Certainly, field experiences support this idea; two classic examples are chick edema disease from exposure to chlorinated dibenzodioxin contamination of feed and horse fatalities from exposure to dioxin contamination of riding arenas.\(^7\)

The fourth characteristic that domestic livestock share with the coalmine canaries is that they can be easily observed. In particular, these observations could be made by adding a chemical exposure component to NADS. The NADS research design permits observations made on a few randomly selected herds in any given State to be extrapolated to all herds in that State through the use of inferential statistics. Through this research design, NADS can provide accurate estimates of the prevalence, incidence, trends, and economic impact of infectious diseases in U.S. domestic livestock. Not surprisingly, this same design could also provide accurate estimates of the prevalence, incidence, and trends of animal exposures to hazardous chemicals and the morbidity caused by such exposures.

The logistics of adding a chemical exposure component to NADS would be straightforward. Federal, State, and university veterinarians, who already visit livestock production units to interview producers and observe livestock, could also collect serum and urine specimens from the livestock. These specimens would then be analyzed both for chlorinated hydrocarbons, such as polychlorinated biphenyls (PCBs) and pesticides, and also for heavy metals, such as lead and cadmium. The results of these analyses would then be mapped and compared with maps of known or suspected areas of chemical contamination. Given our limited and fragmented knowledge of where environmental contamination exists, the results of the livestock specimens are likely to identify new areas for environmental investigation.

Combining the results of the chemical analyses with data from the NADS questionnaire would also permit studies of environmentally induced animal morbidity. For example, if the results of serum PCB analyses of cattle in a given State identify two or more categories of herds of the
same size that differ significantly in their mean PCB levels, these herds could then be classified accordingly and their reproductive health histories compared. If herds with higher serum PCB levels are found to also have higher incidences of infertility and other adverse reproductive effects, a strong association between PCB exposures and poor reproductive performance may be established. One of the benefits of coupling chemical analyses of animal specimens with NADS questionnaire data is that the questionnaire data would also provide extensive information on possible confounders — such as the herds’ histories of brucellosis and infectious bovine rhinotracheitis. Clearly, identifying an association between animal exposures to environmental contaminants and animal morbidity can only be done correctly if such confounders can be ruled out. Because of the type of information that NADS collects, sound epidemiologic decisions could be made regarding the extent to which various animal health outcomes are associated with chemical exposures. Public health officials thus would have sound field-based biological data upon which to make judgements about human health hazards likely to be associated with environmental chemical exposures.

A remaining question to be answered is “What does this new use of NADDS have to offer the individual producer?” The answer is the ability to know whether his or her herd problems or those of a neighboring producer might be caused by exposure to environmental contamination. When a producer has a question like the above now, finding a satisfactory answer is extremely difficult and frequently impossible. Environmental exposures tend to occur at low doses and over long periods. They may also occur intermittently and may, therefore, be difficult to verify by analyses of air, soil, or water samples. Toxicologic data in the scientific literature usually only describe what happens when animals receive high dose exposures for relatively short periods of time. By incorporating chemical exposure surveillance into NADS, veterinarians would be able to tell producers if their herds’ serum or urine chemical levels are higher than the background levels for herds in their States and surrounding States. Veterinarians would also be able to tell producers what NADs would have found about the levels of exposures that are associated with disease.

In summary, for the reasons described above, the Center for Environmental Health, Centers for Disease Control, is excited about collaborating with USDA, State agencies, and universities in this new use for NADS. We look forward to providing a valuable service jointly to the public at large, to State and local health departments, and to the livestock producers of America.

REFERENCES
NATIONAL ANIMAL DISEASE DETECTION SYSTEM
EVALUATION OF LIST FRAMES FOR DISEASE SURVEILLANCE OF CALIFORNIA SWINE

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From the Department of Epidemiology and Preventive Medicine (Gardner, Hird) School of Veterinary Medicine, University of California, Davis, 95616 and the Animal Health Branch, California Department of Food and Agriculture (Heron) Sacramento, California, 95825.

SUMMARY

In this study, we evaluated list frames for disease surveillance sampling of California swine herds. A telephone survey was used to document herd sizes, production characteristics, reproductive performance, use of veterinary services, disease histories and internal parasite control practices of Tulare county pork producers. 96% of confirmed producers on a composite master list frame participated in the survey. Although the Statistical Reporting Service (SRS) list frame included only 54% of surveyed herds and 86% of the breeding swine within the county the distribution of herd sizes, production methods and disease histories closely paralleled those of the entire population of surveyed herds. Subsequently we estimated by area frame sampling that the master list frame accounted for only 28% of the herds with breeding pigs, but over 91% of the swine in the county. The implications of these findings for sampling California swine are discussed.

INTRODUCTION

Statistically valid estimates of disease prevalence, incidence and economic cost are among the ultimate objectives of a well planned animal disease surveillance system such as the National Animal Disease Detection System (NADDS).1 As part of the pilot NADDS program, sampling methodology is being developed to ensure that these objectives are achieved.

List frame, area frame and multiple frame sampling are under consideration as possible sampling methods for the California program. Although area frames are the only complete frames available, they are not particularly efficient alone for livestock estimation,2 nor are they likely to be efficient for disease estimation. List frames of producers, although difficult to develop and almost impossible to keep up to date permit cheaper data collection methods and improved sampling precision. In addition, list frames may include herd size data, an important stratifying variable for diseases studies. Because none of the available livestock list frames in California was designed specifically for disease surveillance, one of the initial steps we took was to evaluate existing lists in this new context.3

The Statistical Reporting Service, US Department of Agriculture (SRS)
has constructed and maintains a master list of California farm and ranch operators for use in multiple frame probability surveys. The master list is updated annually from outside sources, priority being given to crops and livestock of greatest state and national importance. The master list frame contains historic data on livestock inventories and this data forms the basis for stratification of survey samples. Each year operations with swine are selected from the California master list for participation in the December Hog and Pig Survey. Because the SRS list frame was primarily constructed to assist in forecasting crop and livestock production, its potential suitability for disease surveillance needs to be assessed.

In this study we conducted a telephone survey of swine producers in Tulare county, California. Our primary objective was to compare herd sizes, production characteristics, reproductive performance, usage of veterinary services and disease histories of swine belonging to producers on the SRS list frame with those on a composite master list frame. Using area frame sampling we also wanted to determine how many swine producers and breeding pigs had not been included on the composite master list frame.

MATERIALS AND METHODS

Tulare county was selected for the study because it is the largest swine producing county in California, representing approximately 4% of the state's swine herds, but 20% of its pigs. The county is located in the San Joaquin Valley of South Central California and had an estimated pig population of 24,891 pigs in January 1984.

A master list frame was compiled from the following lists of producers:
1. Statistical Reporting Service (SRS) — 50 names
3. Tulare county farm advisor (TCFA) — 14 names
4. California Pork Producers Association (CPPA) — 33 names
5. Feed miller (FM) — 29 names

Producers' names often appeared on more than one list. After duplications from list overlap were removed, 84 names of potential producers remained on the master list. Each producer was mailed a letter in August 1984 outlining the reasons for the survey. A list of questions which would require records to complete was also included with the letter. Producers were phoned by the senior author between September and December 1984 and the survey of 26 questions was administered to producers who had raised at least 1 pig during the period July 1, 1983 to June 30, 1984. The questions covered operation types, herd sizes, housing, sales of pigs, reproductive performance, preweaning mortality, usage of veterinary services, disease histories and internal parasite control practices. The source of each producer's name was kept confidential.

Area frame sampling

Tulare county valley was divided into 230 blocks of approximately 4-6
square miles each. County roads marked on the Tulare county map were used as boundaries for the blocks. After the blocks were sequentially numbered from 1 to 230, a random sample of 18 blocks was drawn using a table of random numbers. Visits were made to rural residences within these blocks between December 12 and December 18, 1984 to determine whether the occupants had swine on their properties. Information collected when the owner was present included the number of pigs and the type of operation. The owner's name and address were checked for inclusion on the composite master list frame. Return visits were not made to properties from which the owner was absent nor were visits made to properties where guard dogs prevented entry. Residential areas present in 6 of the blocks were omitted from the sample (towns of Cutler, Porterville, Terra Bella, Pixley, Farmersville and Exeter).

Statistical Analysis

The data were entered into a computer file and were analyzed using BMDP statistical programs.6 Descriptive statistics were computed for Statistical Reporting Service (SRS) herds, for herds whose names did not appear on the SRS list but were included on any of the other lists (non SRS) and for all Tulare county herds. Inferential statistics were not carried out for the telephone questionnaire results as the survey was essentially a census of all herds on the master list. For dichotomous variables the hypergeometric distribution7 was used to determine the exact probabilities of obtaining a sample from the master list frame with the outcomes we observed in the SRS herds. Reproductive statistics were calculated for 26 herds which provided complete information for the 12 month period to June 30, 1984.

RESULTS

Fifty two of the 84 (61.9%) potential producers replied that they had kept pigs during the year of interest. Of these 52, 50 participated in the survey. Two producers declined to participate because they spoke poor English. The proportion of potential producers confirming that they had pigs by list frame was lowest (56%) for the SRS frame and highest (90%) for the BAH list (Table 1). Response rates to individual questions were highest for questions on herd sizes, operation type, usage of veterinary services, disease histories and parasite control practices (94-100%). The lowest response rates were for questions on numbers of pigs born live and dead, and preweaning mortality rates (60-65%).

Table 2 and figure 1 present the distributions of type of production and herd sizes for SRS, non SRS and all county herds. The SRS list contained more producers classified as farrow to finish (54%) than the non SRS list frame (30%). 40 of the 50 owners reported having breeding pigs during the year. At July 1, 1984 the total number of sows and boars in the surveyed herds were 5068 and 286 respectively. The median number of breeding pigs (entire male and female pigs over 8 months of age) for SRS herds was 21 compared to 18.5 for all Tulare county herds.
The reproductive performance of SRS herds and non SRS herds were similar (Table 3). Overall, the median numbers of pigs born and weaned during the year July 1, 1983 to June 30, 1984 were 10.2 and 8.2 respectively. The preweaning mortality rate was 20.4%.

Thirty eight percent of producers responding to the survey recorded information on disease problems. The type of information recorded usually related to problems in the farrowing house such as mastitis-metritis-agalactia and neonatal scours. Recording of disease events occurred more frequently in herds with more than 50 sows (76.4%) than in herds with 50 or less sows (16.7%). However, rates of disease recording in SRS herds were similar to rates for all producers in Tulare county. Health problems were reported by 23 of 49 (46.9%) producers responding to that question. Herds with more than 50 sows reported problems more frequently (72.2%) than did owners of herds with 50 or less sows (32.3%). The frequency of reporting of ill health by SRS producers did not differ from the population rate. Scours, pneumonia and atrophic rhinitis were the 3 most frequently reported diseases (table 4).

Veterinary services were used infrequently by survey participants (figure 2). Veterinarians visited only 28 of the 50 (56%) producers during the year making a median of 1 visit to each producer. The most common reasons given by owners for requiring veterinarians to visit their ranch were to investigate disease outbreaks, to blood test pigs to meet regulatory requirements, and for surgical procedures such as hernia or prolapse repair.

Internal parasite control practices (table 5) were similar for SRS and all Tulare county herds.

Area frame sampling

Pigs were located on 22 rural holdings in the areas sampled. Three of these operations were already included on the existing list frames. Of the 19 newly identified operations, 8 had breeding swine and 11 fattened pigs. The largest herd not on our existing master list frame had 20 breeding pigs. The median number of breeding pigs in the newly located operations was 2 compared with 17 for the 3 herds we had previously accounted for.

DISCUSSION

No single ideal list frame existed for disease surveillance sampling of Tulare county swine. None of the existing list frames included all population units nor were producers included on the lists in a random fashion. However, the results of our telephone survey suggests that, in Tulare county at least, producers on the SRS list frame are not unrepresentative of production methods, herd sizes, usage of veterinary services or internal parasite control practices of all Tulare county herds. In addition, reported disease histories were also similar. In our questionnaire, we relied on owner perception of disease importance. Thus our results may not have accounted for differing owner preception rates or for differences in disease incidence or prevalence within herds. The low response rate (65%) to
questions on reproductive performance was disappointing. However, it was interesting that the 8 largest swine producers all provided complete information. Five of these 8 producers had computerized record keeping systems which facilitated retrieval of the required information. In smaller herds, the low response rate could be attributed to owners having either insufficient records or being unwilling to analyze existing data.

Despite having the attractive feature of data on herd size, two potential disadvantages of using the SRS list exist. First, because California is a minor swine producing state contributing little to national estimates of production, updating of the SRS master list frame from outside sources such as the California Pork Producers Association is undertaken infrequently. Consequently, producers entering the swine industry may not be included on the SRS list for some time unless they are identified in an alternate survey such as the crop and acreage survey which includes questions on livestock inventories. If the SRS list frame is used for disease surveillance sampling, veterinarians may prove helpful by suggesting outside list sources which could be used to increase the completeness of the SRS list frame. For example, the addition of California Pork Producers Association names to the SRS list would have increased coverage to 82% of the master list frame. The more complete the SRS list frame, the less likely that the sampled population and the target population will differ. The low confirmation rate (56%) for producers whose names appeared on the SRS list frame means that a larger sample than normally required will need to be drawn to account for reduced enrollments. This situation arises because SRS may keep operators names on its master list despite failure by the operator to reply to surveys for up to 3 years.

Area frame sampling showed that our Tulare county master list frame missed a large number of small herds. We randomly sampled an area representing approximately 7.8% of the county and found 3 of the 52 (5.8%) herds on our master list frame. This suggests that herds on the master list frame were distributed homogeneously throughout the county. By expansion, the estimated number of breeding pigs not included on our master list frame is 525 (8.9% of the county's swine). Furthermore, we speculate that there are 102 breeding herds not included on our master list frame. Thus the master list frame accounted for 28% of the herds with breeding pigs but over 91% of the county swine. Our projections may overestimate the true coverage of the master list frame as we omitted 6 residential areas. Nevertheless, it is highly unlikely that we would have missed any large herds by omitting these areas.

The omission of area frame herds from the Tulare master list is unlikely to be important for endemic disease surveillance. Most of the omitted herds were either small backyard producers raising a few pigs for family consumption or participants in high school swine projects. We believe that their exclusion from list frames is not detrimental because NADDS is primarily interested in estimation of disease rates and economic costs in commercial herds.
Although SRS uses the same procedures to update lists statewide, the results we obtained in Tulare county may not apply to the rest of California. Despite the potential disadvantages outlined, the SRS list frame appears to be the most attractive sampling frame for future disease surveillance of California swine.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of G. Tucker and R. Bosecker, Statistical Reporting Service, Sacramento; W. Sischo and K. Parker, University of California, Davis; J. Picanso, California Pork Producers Association; R. Broemmel, Reedley and R. Miller University Cooperative Extension, Visalia.

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REFERENCES

Table 1. Number and proportion of producers participating in the Tulare swine survey (September-December 1984) by the list frame on which they appeared

<table>
<thead>
<tr>
<th>List frame</th>
<th>No. potential producers</th>
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<td>20</td>
<td>18</td>
<td>90.0</td>
<td>18</td>
<td>100.0</td>
</tr>
<tr>
<td>TCPA</td>
<td>14</td>
<td>13</td>
<td>85.7</td>
<td>13</td>
<td>100.0</td>
</tr>
<tr>
<td>CPPA</td>
<td>33</td>
<td>26</td>
<td>78.8</td>
<td>26</td>
<td>100.0</td>
</tr>
<tr>
<td>FM</td>
<td>29</td>
<td>25</td>
<td>86.2</td>
<td>24</td>
<td>96.0</td>
</tr>
<tr>
<td>Master list</td>
<td>84</td>
<td>52</td>
<td>61.9</td>
<td>50</td>
<td>96.1</td>
</tr>
</tbody>
</table>

a Producers may appear on more than one list. List frames: SRS = Statistical Reporting Service; BAH = Bureau of Animal Health; TCPA = Tulare county farm advisor; CPPA = California Pork Producers Association; FM = Feed miller

b Confirmation rate = No. confirmed with swine ÷ no. potential producers x 100%

c Response rate = No. responding to survey ÷ no. confirmed with swine x 100%
Table 2. Distribution of production types by list frame for 50 surveyed swine herds in Tulare County, California (September - December 1984)

<table>
<thead>
<tr>
<th>Production method</th>
<th>SRS&lt;sup&gt;e&lt;/sup&gt; (n=27)</th>
<th>non SRS (n=23)</th>
<th>All herds (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrow to finish&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 (55%)</td>
<td>7 (30%)</td>
<td>22 (44%)</td>
</tr>
<tr>
<td>Feeder/weaned pig producer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4 (14%)</td>
<td>5 (22%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Fattening&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (7%)</td>
<td>2 (8%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Miscellaneous&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6 (22%)</td>
<td>9 (39%)</td>
<td>15 (30%)</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> Minimum of 10 breeding pigs and market weight pigs sold as roasters (90-100 lbs) or hogs (210-240 lbs).

<sup>b</sup> Minimum of 10 breeding pigs and majority of pigs sold as feeders or weaned pigs at 6 - 12 weeks of age.

<sup>c</sup> Minimum of 40 fattening pigs.

<sup>d</sup> Includes FFA, 4H project pigs, herds with less than 10 sows or fattening herds with less than 40 fattening pigs.

<sup>e</sup> List frames: SRS = Statistical Reporting Service, non SRS = composite list of all herds not on the SRS list.
Figure 1. Box and whisker display of total breeding herd size for Statistical Reporting Service (SRS), a composite list of all herds not on the SRS list (non SRS) and all herds in Tulare county, California at July 1, 1984.
Table 3. Reproductive performance of 26 Tulare county, California herds by the list frame on which they appeared for the period July 1, 1983 to June 30, 1984.

<table>
<thead>
<tr>
<th></th>
<th>SRS(^e) (n=12)</th>
<th>non SRS (n=14)</th>
<th>All herds (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median no. pigs born per litter (^a)</td>
<td>10.0</td>
<td>11.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Median no. pigs weaned per litter (^b)</td>
<td>7.9</td>
<td>8.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Median preweaning mortality (%) (^c)</td>
<td>20.3</td>
<td>20.6</td>
<td>20.4</td>
</tr>
<tr>
<td>Median litters per female per yr (^d)</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\(^a\) No. pigs born per litter = 100% of pigs born live or dead
\(^b\) No. pigs weaned per litter = 100% of pigs weaned
\(^c\) Preweaning mortality % = \(\frac{\text{No. pigs born} - \text{No. pigs weaned}}{\text{No. pigs born}}\) x 100%
\(^d\) Female - any definition given by producer was used in the analysis
\(^e\) List frames: SRS = Statistical Reporting Service

non SRS = composite list of all herds not on the SRS list
### Table 4. Disease histories for swine herds in Tulare county, California for the year July 1, 1983 to June 30, 1984 by the list frame on which they appeared

<table>
<thead>
<tr>
<th>Disease problem previous yr.</th>
<th>SRS (n=27)</th>
<th>non SRS (n=22)</th>
<th>All herds (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) scours</td>
<td>7 (25.9%)</td>
<td>4 (18.2%)</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td>ii) pneumonia</td>
<td>7 (25.9%)</td>
<td>4 (18.2%)</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td>iii) atrophic rhinitis</td>
<td>4 (14.8%)</td>
<td>2 (9.1%)</td>
<td>6 (12.2%)</td>
</tr>
<tr>
<td>iv) other diseases&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (22.2%)</td>
<td>6 (27.3%)</td>
<td>12 (24.5%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Other diseases reported by producers included parvovirus, internal and external parasites, infertility, swine dysentery and mastitis-metritis-agalactia. None of these diseases occurred on more than 4 ranches.

<sup>b</sup> List frames : SRS = Statistical Reporting Service
non SRS = composite list of all herds not on the SRS list

<sup>c</sup> P values > 0.1
Table 5. Internal parasite control practices of surveyed herds in Tulare county, California by the list frame on which they appeared (September-December 1984).

<table>
<thead>
<tr>
<th>Type of pig treated</th>
<th>SRS(^d)</th>
<th>non SRS</th>
<th>All herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Sows (^a)</td>
<td>18 (85.7%)</td>
<td>18 (90.0%)</td>
<td>36 (87.8%)(^e)</td>
</tr>
<tr>
<td>ii) Boars (^a)</td>
<td>16 (72.7%)</td>
<td>13 (81.3%)</td>
<td>29 (76.3%)(^e)</td>
</tr>
<tr>
<td>iii) Weaned pigs (^b)</td>
<td>20 (74.0%)</td>
<td>18 (81.8%)</td>
<td>38 (77.5%)(^e)</td>
</tr>
<tr>
<td>iv) Finisher pigs (^c)</td>
<td>7 (30.4%)</td>
<td>15 (78.9%)</td>
<td>22 (52.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency of treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Sows per annum</td>
<td>3.3</td>
<td>4.4</td>
<td>3.8</td>
</tr>
<tr>
<td>ii) Boars per annum</td>
<td>4.6</td>
<td>3.2</td>
<td>4.0</td>
</tr>
<tr>
<td>iii) Weaned pigs</td>
<td>1.1</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>iv) Finisher pigs</td>
<td>0.3</td>
<td>1.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^a\) Sows and boars defined as female and entire male pigs over 8 months of age.

\(^b\) Weaned pig defined as a pig of any age from weaning to 100 lbs live weight.

\(^c\) Finisher pig defined as a pig in the weight range 100-250 lbs and less than 8 months of age.

\(^d\) List frames: SRS = Statistical Reporting Service

\(^e\) List frame: non SRS = composite list of all herds not on the SRS list

\(e\) P values > 0.1
Figure 2. Box and whisker display of the number of veterinary visits to Statistical Reporting Service (SRS) herds, herds on a composite list other than SRS (non SRS) and all Tulare county swine herds for the period July 1, 1983 to June 30, 1984.
THE ANIMAL DISEASE SURVEY SAMPLING AND ESTIMATION PROBLEM

Victor C. Beal, Jr., PhD. Chief Staff Biometrician, Veterinary Services, APHIS, USDA, Hyattsville, MD. October 1985.

Two important animal disease surveillance problems involve national animal populations. One problem involves estimating disease levels in a population of animals. The other surveillance problem involves detecting infected herds and flocks. This paper is aimed at the epidemiologist with inadequate training and experience in statistics and at the statistician with inadequate experience in animal disease. It expands upon an earlier one by the author (Beal, 1984) on the contrast in the two problems and the nature of the first problem.

Activities of the Committee on Animal Disease Surveillance (CADS), formerly the Committee on Morbidity-Mortality, of the United States (U.S.) Animal Health Association, and its predecessor committee, the Committee on Morbidity and Mortality Statistics of the U.S. Livestock Sanitary Association, have been aimed at solving the problem of disease rate estimation. CADS activities have aided in initiating pilot projects to develop a National Animal Disease Surveillance (NADS) system as it was first called or a National Animal Disease Detection System (NADDS) as it is now called. NADDS is intended to be a system providing statistically valid estimates of incidence or prevalence. Hence, NADDS is aimed at solving the problem of disease rate estimation.

In contrast, activities of animal disease control or eradication involve detecting and eliminating foci of disease. This includes detecting outbreaks of diseases exotic to the U.S. or to parts of the U.S. and detecting foci of endemic diseases being eradicated. NADDS is not a disease detection system in the historical sense of being effective in detecting introductions of exotic diseases or in detecting foci of endemic diseases being eradicated. Rather, NADDS is a detection system capable of statistically valid estimates of moderate levels of endemic disease. Hence, NADDS should not be considered to be effective in detecting introductions of diseases exotic to the U.S. or parts of the U.S. or in detecting foci of endemic diseases being eradicated.

CONTRAST IN TWO SURVEILLANCE PROBLEMS

Both surveillance problems involve sampling animal populations. However, the contrasting nature of the two problems requires two different approaches in sampling. The science of sampling statistics aids in differentiating the two problems. This science explains why a system like NADDS, which is being developed to estimate the level of disease in a population, is not effective in detecting infected herds or flocks for disease control or eradication and in detecting exotic disease outbreaks. Also, this science explains why a surveillance system developed to detect foci of disease for eradication or control is often of little use in estimating the level of the target disease.
ANIMAL DISEASE SURVEY

Need or non-need for PBR and PPAN: The problem of disease rate estimation of endemic diseases with NADDS or any other system needs a careful probability based random (PBR) sampling of a small fraction of the herds and flocks in the population based upon probability proportional to size (PPS) which in this case is probability proportional to animal numbers (PPAN). In contrast, the problem of disease detection for control or eradication needs intensive surveillance of large parts of the population to detect the disease.

Types of Statistical Estimation in Disease Surveillance

The application of sampling statistics to these two problems may be likened to being statistical estimation. The estimation problem relating to the level of disease involves estimating prevalence and incidence rates. This permits estimating physical loss due to disease. The estimation problem relating to detecting foci of disease involves determining adequate surveillance levels for detecting disease for control or eradication.

Statistical estimation of disease rates: Veterinary Services (VS) of the Animal and Plant Health Inspection Service (APHIS) is involved in NADDS pilot projects in six states to develop procedures to estimate prevalence and incidence rates for diseases endemic in a population. This estimation problem will be discussed in detail later. This problem must involve PBR samples where each member of the population must have a known probability unequal to zero of being in the sample.

Several authors (Beal and Cox, 1970; Beal, 1975, 1980a, 1980b, 1983a, 1983b; McCallon, 1981; McCallon and Beal, 1982; and Leech and Sellers, 1979) have discussed the inadequacy of traditional animal diseases surveillance data in estimating disease levels. They demonstrated traditional animal disease data to be totally inadequate in estimating disease levels.

Systems felt to be able to provide estimates of disease levels have been proposed in the past (Robson and Baker, 1957; Cox and Huddleston, 1971; Diesch et al., 1974, McCallon, 1981; and Diesch et al., 1981). The current NADDS pilot projects are based on similar proposals by McCallon (Sharman, 1980; McCallon, 1981) and Diesch et al. (1981).

As discussed by the author (Beal, 1983b), PBR samples of market animals can provide some information. However, the most important information must come from PBR samples consisting of herds and flocks. Two major statistical problems in estimating incidence and prevalence are to determine what is an adequate sample size and what is an adequate survey design.

Statistical estimation in detecting foci of disease: The estimation problem relating to detecting foci of disease or to the likelihood of the presence of disease has been examined by several workers (Harvey, 1958; Damon, 1961; Beal, 1977, 1983a, and 1983b). Starting in 1963, the author expanded on the earlier work of Harvey and Damon in the Agricultural Research Service (ARS).
The author shows in his *Regulatory Statistics*, Parts III, IV, and VII (Beal, 1983a) the extensive surveillance needed for adequate levels of disease detection for control and eradication. It was obvious that levels of sampling that are adequate for estimating prevalence and incidence are inadequate to detect foci of disease for control or eradication.

Part IV examines the detection of foci of bovine brucellosis and tuberculosis (TB) with the Market Cattle Identification (MCI) program. Principles enunciated in that section apply to other diseases in cattle and to diseases in other species which can be detected at slaughter or at the market.

Part III has principles for the random sampling of animals in herds or flocks to detect disease in those herds or flocks. That section also has tables with required sample sizes for several infection rates and herd sizes. Appendix III has the statistical method used in Parts III and IV. Part VII has additional statistical principles for detecting foci of disease.

**Detecting residue or toxic waste problems:** Part VII also discusses the inadequacy of samples of limited size in detecting residue or toxic waste problems. The example in Part VII involves detecting a Hexachloro-Benzene (HCB) environmental problem by the residue sampling of slaughter animals in Louisiana in December 1972. This problem was found with a nationwide sample of 300 animals. The conclusion must be drawn that there must be many other environmental residue problems in the U.S. going undetected at the sampling rates that are often involved and will be involved nationally with NADDS.

**NADDS in statistical estimation:** Hence, as stated above, NADDS should not and must not be considered to be effective in detecting the introduction of diseases exotic to the U.S. or parts of the U.S. or in detecting foci of endemic diseases being eradicated or in detecting potentially serious environmental and toxic waste problems. Rather, NADDS is a system capable of statistically valid estimates of disease existing at endemic levels in the United States.

**BASIC NEEDS IN ESTIMATING DISEASE RATES**

Two basic needs in estimating disease incidences and prevalence are statistical and diagnostic validity. Discussions of the nature of an adequate system for estimating incidence and prevalence have tended not to properly recognize the importance and nature of these two needs.

**Diagnostic validity:** Diagnostic validity involves two aspects. They depend upon whether or not diagnostic samples are obtained. Without diagnostic samples, diagnostic validity consists of clinically valid descriptions of the disease being observed. With diagnostic samples, diagnostic validity consists of accurately relating clinical observations to laboratory results.

A complete achievement of either aspect of diagnostic validity needs veterinary involvement. Some groups have proposed that non-veterinary enumerators could obtain the needed data more cost-effectively than
veterinarians could. However, some past experiences cast doubt on this idea. These experiences include a pilot disease survey project in Iowa in 1946-47 (Anonymous, 1947 and Snedecor, 1947). In that study, three groups of farms were surveyed by employees of a market research company while a fourth group of farms was surveyed by a former military veterinarian. The conclusion was that the veterinarian obtained more valid data than the professional interviewers due to his knowledge of animal diseases and livestock conditions.

Statistical validity: Statistical validity is paramount to valid disease rate estimation and needs valid survey design and adequate sample size. As noted above, statistical validity also needs PBR herd selection and PPAN herd assignment. Also, statistical validity needs accurate data recording. This means that diagnostic validity is a part of statistical validity in addition to being a separate factor.

The problem of statistically adequate sample sizes in disease estimation has been related to sample sizes used for the Statistical Reporting Service's (SRS) livestock estimates. Samples of 1,000 to 1,500 herds have been used to obtain adequate statewide estimates of livestock inventory. However, it will be seen that smaller numbers will suffice for animal disease estimates.

In addition, adequate survey design is of concern. While adequate sample size is a part of survey design, survey design is considered here as to how the sample relates to the population of interest. Therefore, it is desirable to discuss various aspects of animal disease estimation. In doing so, past and present experiences in the field of animal disease are used.

PBR sample need: The need for PBR samples needs emphasis. As noted above, each unit of interest in the population must have a known probability unequal to zero of being selected. If disease estimates are needed for a group of states or for the U.S., the need for a known probability applies to each state in the group of states or in the U.S., and to each herd or flock in each state. If the herd or flock is selected from a county rather than from the entire state, this need for a known probability applies to each county in the state and to each herd or flock within a county.

Unless PBR sampling is done, haphazard sampling occurs and accuracy of a survey estimate cannot be computed. If specific states or herds are selected because they supposedly are like other states or herds which are excluded, there is no idea if incidence or prevalence are overestimated or underestimated while with a PBR sample of an adequately known population, a true estimate is obtained. The contrast between adequately and inadequately known populations is related later to trichinae in garbage fed swine and salmonella in feed.

PPAN sample need: The need for PPAN assignment of herds to be selected also needs emphasis. Variation among herd size strata, among states, within states, and among herds within the various strata are major factors in animal disease differences. The most accurate national and statewide estimates of prevalence and incidence need PPAN herd as-
Assignment among herd size strata, among states, and within states since this maximizes accuracy for a given sample size.

SURVEY DESIGN AND SAMPLE SELECTION

Survey design and sample selection are important to survey validity. Survey design must consider the population structure. Sample selection must reflect differences in population structure and knowledge of the population.

Factors in Survey Design

Some important factors will be considered in survey design. They are herd size differences, differences within or among states, and differences in husbandry.

Herd size importance: Experience shows herd size to be important in survey design. Theoretical and empirical evidence for brucellosis and empirical evidence for pseudorabies exists. These examples show the importance of herd size in disease differences. Many domestic diseases involve animal-to-animal spread. Herds with a greater number of herd additions have a greater chance of becoming infected with larger herds tending to have more herd additions.

Therefore, one cannot extrapolate disease rates obtained from herd surveys to the national herd unless herd size is considered. Also, herd size must be considered in survey design in order to make sure that an adequate number of large herds are obtained for estimation purposes.

Herd size difference in brucellosis: The author (Beal, 1983c) showed herd size to be important for brucellosis theoretically with a mathematical simulation model. The importance was shown empirically in a study of brucellosis program data for 1976 and 1978 (Amosson, 1978 and 1981). A greater proportion of large herds were infected than small herds. In the case of the empirical data, large herds tended to have a lower animal infection rate within the herd than small herds. These differences relate to animal replacement policies, husbandry practices, and animal density within the herd.

Herd size difference in pseudorabies: Pseudorabies evidence is empirical. This is shown with data from Purdue University (Gustafson and Scherba, 1978; McCallon and Beal, 1982). Starting about 1974, the disease seemed to be increasing in prevalence and economic importance. Therefore, a mail survey of National Pork Producers Council members was done in order to gain data on prevalence and losses (Gustafson and Scherba, 1978). Despite well-known inadequacies of mail surveys as discussed by authors such as Cochran (1963), the survey provided useful information.

However, in estimating economic loss, herd size was ignored in expanding the survey data to the U.S. population of swine sold. Therefore, losses were overestimated by 100 percent due to the resulting failure to stratify the data by herd size. Tables 1 through 3 have results of the survey and their expansion to the U.S. population of swine sold.
**Table 1.** Pseudorabies Survey of National Pork Producers Council Members—
Number of Herds and Number of Animals Sold by Herds in the Survey.

| Type of Swine Sold | Number of Herds | Number of Animals Sold From | Herd Infection Status | | |
|--------------------|-----------------|----------------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                    | Infected | Uninfected | Total | Infected | Uninfected | All |
| Commercial         | 74       | 1,983     | 2,057 | 105,890 | 1,899,740 | 2,005,630 |
| Feeder             | 13       | 529       | 542   | 23,860  | 403,861   | 427,721   |
| Seed-Stock         | 8        | 243       | 251   | 4,800   | 75,908    | 80,708    |
| Total              | 81       | 2,340     | 2,421 | 134,550 | 2,379,509 | 2,514,059 |

* Herd numbers do not sum to total since some herds sold two or more types.

**Table 2.** Pseudorabies Survey—Average Number of Animals Sold Per Type of Animal and for Entire Herd.

<table>
<thead>
<tr>
<th>Infection Status</th>
<th>Hogs Sold</th>
<th>Hogs Sold per Group</th>
<th>Hogs Sold per Sale Type</th>
<th>Ave. Hogs per Herd</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Herds</td>
<td>2,514,059</td>
<td>2,850</td>
<td>882</td>
<td>1,038</td>
</tr>
<tr>
<td>Uninfected Herds</td>
<td>2,379,509</td>
<td>2,755</td>
<td>864</td>
<td>1,017</td>
</tr>
<tr>
<td>All Infected Herds</td>
<td>134,550</td>
<td>95</td>
<td>1,416</td>
<td>1,661</td>
</tr>
<tr>
<td>Loss Data Reported</td>
<td>97,910</td>
<td>52</td>
<td>1,605</td>
<td>1,883</td>
</tr>
<tr>
<td>Loss Data Unreported</td>
<td>36,640</td>
<td>29</td>
<td>1,263</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Expansion of Swine Sold from Herds Reporting Loss Data in Pseudorabies Survey to U.S. Population of Swine Sold.

<table>
<thead>
<tr>
<th>Number of Swine Sold Per Herd</th>
<th>Number of Swine in Survey Grouped According to Sales Class</th>
<th>Percent in Loss</th>
<th>Number of Swine Sold in U.S. From Loss Type Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sales Class</td>
<td>Actual Loss</td>
<td>Data Herds</td>
</tr>
<tr>
<td></td>
<td>Herd Groups</td>
<td>Data Herds</td>
<td></td>
</tr>
<tr>
<td>1 to 199</td>
<td>47,394</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>200 to 499</td>
<td>242,291</td>
<td>2,710</td>
<td>1.118</td>
</tr>
<tr>
<td>500 to 1,000</td>
<td>457,813</td>
<td>10,300</td>
<td>2.250</td>
</tr>
<tr>
<td>1,000 to 1,999</td>
<td>627,080</td>
<td>15,600</td>
<td>2.488</td>
</tr>
<tr>
<td>2,000 to 2,999</td>
<td>301,880</td>
<td>12,500</td>
<td>4.114</td>
</tr>
<tr>
<td>3,000 to 4,999</td>
<td>227,200</td>
<td>28,000</td>
<td>12.324</td>
</tr>
<tr>
<td>5,000 and Over</td>
<td>610,400</td>
<td>28,800</td>
<td>4.718</td>
</tr>
<tr>
<td>Total</td>
<td>2,514,058</td>
<td>97,910</td>
<td>79,897,397</td>
</tr>
</tbody>
</table>

* Straight Line Estimate = (97,910)/(2,514,058) x 79,897,397 = 3,111,604.
  
  Straight Line Estimate is in Error by 99.7 Percent.

* Data from 1974 Ag. Census was for 2,000 to 4,999 head sold. Number sold for the two groups was interpolated with probability x 3-log graph paper.
Table 1 has a summary of the survey data. Animals sold were enumerated for three classes, namely commercial, feeder, and seed-stock swine. As expected due to the above noted experiences with bovine brucellosis and to experiences with bovine TB and as shown in Table 2, the survey data showed larger herds more likely to be infected. The average size of an infected herd was 1,661 swine sold and the average size of an uninfected herd was 1,017 swine sold. The table has data on swine sold per herd and per sales class.

The difference in herd size between infected and uninfected herds required an expansion of survey data to the U.S. population of swine sold on the basis of herd size rather than with a straight-line expansion. Therefore, the authors of the survey report provided VS with basic data to permit an expansion based upon herd size distribution.

Loss data was reported for 52 infected herds while loss data was not reported for the other 29 infected herds. Data for infected herds reporting loss data was available for the entire herd and by type of animal sold. However, the only data available for individual uninfected herds was a computer list with data for each type of sale and not for the entire herd. The computer list had all herds in the survey including infected and uninfected herds by herd size for the three types of swine sold.

Therefore, expansions from the survey population to the U.S. population of swine sold as shown in Table 3 had to be based on swine sold per class of swine rather than per herd. Data on swine sold in the U.S. for the 1974 U.S. Census of Agriculture is also shown in Table 3.

With herd size not considered, the estimate of animals sold from infected herds is 3,111,604 head for the United States. With herd size considered, a lesser estimate of animals sold from infected herds is 1,558,212 head for the United States. The U.S. is fortunate that reliable data is available for herd size. This data should be used, when applicable, in estimating prevalence.

Survey design and differences among states: Differences in disease rates among states exist for many diseases. An example is anaplasmosis in the case of a national PBR sample survey completed in 1973. This survey used brucellosis MCI blood samples. Results from the survey showed large differences in prevalence rates among states.

This data was compared with data from the defunct National Report of Animal Diseases (NRAD). This showed that data from a traditional reporting system, such as NRAD, consisting of practitioner questionnaires and laboratory tests cannot provide estimates of relative prevalence among states. Table 4 (Beal, 1980b) had data from NRAD and from the U.S. survey. Arkansas had 36 percent and California had less than 1 percent of the anaplasmosis reported for the U.S. in NRAD. However, the survey showed 5.3 percent for Arkansas and 10.4 percent for California. Similar discrepancies are seen for other states. Results such as these provided much of the impetus for the NADDS pilot projects.
Survey design and differences within states: Differences in disease rates within a state can be cited for various diseases. An example is anaplasmosis in Arkansas and Missouri. The 1973 anaplasmosis survey showed geographically related differences in these states. Differences in anaplasmosis prevalence within these states were found to be correlated with the location of the Mississippi, Missouri, and Arkansas rivers.

Survey Design and NADDS

Due to past findings such as those of pseudorabies and brucellosis, herd size stratification is an important component of the NADDS pilot projects. However, some other aspects of proper design have been ignored. An important example is the relationship of herds sampled to animal numbers within a state.

Some of the participants at the early NADDS training courses pointed out to the author that it is a serious error to ignore the relationship of animal numbers or PPAN to the number of herds selected. These participants questioned whether accurate extrapolation to the population from the sample could be done if the herds to be sampled were not selected with regard to PPAN. This aspect of PPAN is especially important due to such considerations as those cited above for anaplasmosis.

However, as noted then, it was more important at that time to get as much information as possible on problems of data collection than to have data that could be accurately extrapolated to the population. This required the participation of as many Veterinary Medical Officers (VMO) as possible. This was best done with the assignment of an equal number of herds per VMO.

However, in the second round of data collection, it is important to obtain data that can be expanded to the population with as much accuracy as possible. This requires herd assignment on the basis of PPAN rather than VMO availability. Thus, if one-half of the swine in a state is in one VMO section, one-half of the swine herds selected should be from that VMO section.
Sample Selection in NADDS

Sample selection is also important to accurate expansion of data from a survey to the population of interest. As stated above, a basic requirement is that each herd in the population must have known probability unequal to zero of being selected for the survey. Various methods of selection have been tried for the NADDS pilot projects. Some past experiences in VS are described later.

The herds sampled from the population can be obtained from lists of herds or from geographical areas. The use of lists is selection from a list frame. Selection from an area is selection from an area frame. Multiple frame selection and estimation refers to the use of both types of sampling frames in one survey. All three methods of selection have been tried in the NADDS pilot projects. Experiences of the NADDS participants in PBR sample selection have been important in deciding what alternatives would be tried.

List frame selection: PBR herd selection from lists was done in Ohio and Tennessee. A satisfactory use of list frames requires complete herd lists. However, such lists were not available and each VMO had to make a list. Even with existing SRS lists, small herds are underrepresented. This is especially the case with lists made from the records of county extension agents and other records available to the VMO. Therefore, this method of PBR herd selection was unsatisfactory for the NADDS pilot projects.

Area frame selection: Therefore, area frame selection was used for the Iowa pilot project. Random paths were drawn on road maps and the first herd of the correct herd size was taken. These paths are called random walks. This proved unsatisfactory in selecting large herds. Also, even for small herds, the probabilities of selection could not be determined due to an unequal amount of farm frontage on the various roads. This became evident when the same method was tried in Colorado.

Multiple frame selection: Multiple frame selection was used in Colorado. There were complete lists of herds for milk cows, quarantined feed lots, and sheep shorn. Hence, lists were used for these livestock classes. Multiple frame sampling was used for beef cow herds.

If a small number of beef herds was indicated by SRS records for a specific herd size, a list was made for that county and herd size. A small number of herds was construed to be about 20 herds in the county. If a large number of herds was indicated by SRS records for the herd size and county, then selection was done from area segments equal to a density of one herd per area segment. Tables 5 and 6 show the specific counties in Colorado, herd sizes, and number of herds indicated for the county by SRS records. Table 5 has the herds selected from lists and Table 6 has the herds selected from an area.

An experience with the random road path or random drive in Weld County showed the random walk method of selection to be invalid. The first area selected was in the middle of a national grassland. The random
### Table 5. Data on Beef Cow Herds Sampled from a List for Colorado.

<table>
<thead>
<tr>
<th>Herd Size</th>
<th>County</th>
<th>Number of Herds</th>
<th>Sample method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 +</td>
<td>Mesa</td>
<td>12</td>
<td>List</td>
</tr>
<tr>
<td>500-999</td>
<td>Mesa</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>1,000 +</td>
<td>Jackson</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>500-999</td>
<td>Routt</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1,000 +</td>
<td>Weld</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>1,000 +</td>
<td>Yuma</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1,000 +</td>
<td>Lincoln</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>500-999</td>
<td>Kit Carson</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>1,000 +</td>
<td>Conejos</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>200-499</td>
<td>Alamosa</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1,000 +</td>
<td>Prowers</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>200-499</td>
<td>Otero</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>500-999</td>
<td>Gunnison</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>200-499</td>
<td>Gunnison</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>200-499</td>
<td>Jackson</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6. Density Per Square Mile of Beef Herds Sampled for NADDS in Colorado.

<table>
<thead>
<tr>
<th>County</th>
<th>Sq. Mi. Area</th>
<th>Herd Size</th>
<th>Number of Herds</th>
<th>Sq. Mi. Per Herd</th>
<th>Mi. Sq. Per Herd</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arapahoe</td>
<td>820</td>
<td>10-199</td>
<td>113</td>
<td>7.26</td>
<td>2.7</td>
<td>2 x 2</td>
</tr>
<tr>
<td>Elbert</td>
<td>1,864</td>
<td>10-199</td>
<td>262</td>
<td>7.11</td>
<td>2.7</td>
<td>2 x 2</td>
</tr>
<tr>
<td>Larrimer</td>
<td>2,619</td>
<td>200-499</td>
<td>252</td>
<td>50.37</td>
<td>7.1</td>
<td>4 x 4</td>
</tr>
<tr>
<td>Logan</td>
<td>1,827</td>
<td>10-199</td>
<td>426</td>
<td>4.29</td>
<td>2.1</td>
<td>2 x 2</td>
</tr>
<tr>
<td>Otero</td>
<td>1,267</td>
<td>10-199</td>
<td>214</td>
<td>5.92</td>
<td>2.4</td>
<td>2 x 2</td>
</tr>
<tr>
<td>Weld</td>
<td>4,004</td>
<td>200-499</td>
<td>225</td>
<td>17.80</td>
<td>4.2</td>
<td>4 x 4</td>
</tr>
<tr>
<td>Yuma</td>
<td>2,383</td>
<td>10-199</td>
<td>435</td>
<td>5.48</td>
<td>2.3</td>
<td>2 x 2</td>
</tr>
</tbody>
</table>

The path obtained was obviously inadequate and the likelihood was that there would be no herds along the random path drive. Therefore, one of the Colorado epidemiologists suggested that random circles equal to the average area for one herd be selected. It was then decided to select random squares equal to one herd per square.

Table 6 had data needed for the specific counties in Colorado. An area equal to about one herd per square was computed. Six of these areas were selected for each herd needed in a county. Each square was examined in turn until a herd of the proper size was found. If more than one herd was found in an area, a list was made of the herds and a herd was randomly selected from the list.

Data for Iowa was used to compute the area sizes that would have been used in that state. Table 7 has some of that data. Iowa is laid out in townships and sections. Therefore, areas were designated that would be in accordance with sections within townships. It is seen that the average number of square miles per herd is very great in some cases. Those herds would be selected from a list in accordance with the multiple frame concept.
Table 7. Density Per Square Mile of Herds Sampled for NADDS in Iowa.

<table>
<thead>
<tr>
<th>County</th>
<th>Area in Sq.Mi.</th>
<th>Type of Herd</th>
<th>Number of Herd of Size</th>
<th>Per Sq.Mi.</th>
<th>Per M.Sq.</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buena Vista</td>
<td>575</td>
<td>Hogs</td>
<td>1,000+</td>
<td>37</td>
<td>15.54</td>
<td>3.9</td>
</tr>
<tr>
<td>Osceola</td>
<td>399</td>
<td>Feeder</td>
<td>10-199</td>
<td>168</td>
<td>2.38</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>399</td>
<td>Milk</td>
<td>50+</td>
<td>4</td>
<td>99.75</td>
<td>10.0</td>
</tr>
<tr>
<td>Sioux</td>
<td>769</td>
<td>Hogs</td>
<td>500-999</td>
<td>181</td>
<td>4.25</td>
<td>2.1</td>
</tr>
<tr>
<td>Franklin</td>
<td>583</td>
<td>Feeder</td>
<td>200-499</td>
<td>27</td>
<td>21.59</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cow/Calf</td>
<td>200+</td>
<td>10</td>
<td>58.3</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hogs</td>
<td>300-499</td>
<td>81</td>
<td>7.20</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hogs</td>
<td>1,000+</td>
<td>33</td>
<td>17.67</td>
<td>4.2</td>
</tr>
<tr>
<td>Black Hawk</td>
<td>573</td>
<td>Feeder</td>
<td>1,000+</td>
<td>4</td>
<td>143.25</td>
<td>12.0</td>
</tr>
<tr>
<td>Howard</td>
<td>473</td>
<td>Milk</td>
<td>10-49</td>
<td>171</td>
<td>2.77</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hogs</td>
<td>100-299</td>
<td>121</td>
<td>3.91</td>
<td>2.0</td>
</tr>
<tr>
<td>Mitchell</td>
<td>470</td>
<td>Hogs</td>
<td>500-999</td>
<td>63</td>
<td>7.46</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Probability of Herd Selection

Probabilities of the selection of a herd with above method were computed with the Poisson probability function. A consistent relationship was found between the probability of selection and the density of herds per area in the range of 1/8th herd to 4 herds per area.

The Poisson in animal disease and health: The Poisson probability function is commonly used in animal and human disease and health problems. It may be used in its own accord as is the case here, or it may be used to approximate the traditional one-variable binomial function. It has become evident to the author that some epidemiologists in animal and human health do not understand its use to approximate the one-variable binomial.

This became evident during the course of the author's work in modeling animal disease spread with the double and triple binomial system which has two or more variables (Beal 1980a and 1983c). These epidemiologists stated that "... the probability of a herd becoming infected through inter-herd movement of infected animals is Poisson in nature rather than binomial." These epidemiologists had been introduced to the Poisson in approximating the one-variable with characteristics such as cancer rates.

This statement about the Poisson shows that these epidemiologists did not understand that the Poisson was being used to approximate the one-variable binomial and that the one-variable binomial is not the function of choice in modeling animal disease spread by the inter-herd movement of infected animals.

Poisson defined: Figure 1 shows the Poisson probability function and defines the variables involved. The definition gives the population unit being sampled. The population unit being sampled could be a cubic foot of soil in sampling for insects in the soil, the area of a slide in sampling for bacteria, or a specified area of some size in sampling for cattle herds.
The definition also gives the average number of items found in the population unit being sampled. The average number could be the average number of insects per cubic foot of soil, the average number of bacteria on an area of a slide, or the average number of cattle herds in a specified area. The definition gives "X" as the number of items found in the population unit being sampled. This could be the number of insects observed in a sample of a cubic foot of soil or the number of herds found in the specified area.

Finally, the definition gives the formula for calculating the probability of observing a specific number "X" of items in the population unit that is sampled when there is a certain average number of items expected.

**Poisson in examining probability of herd selection:** Table 8 has an example of the average number of herds varying from \( \frac{1}{8} \) herd to 4 herds per area of interest. The table shows the probability of finding "X" number of herds in the area sampled with "X" ranging from 0 to 8.

<table>
<thead>
<tr>
<th>Number of Herds in Area</th>
<th>Probability of Given Number of Herds in Randomly Selected Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td>0</td>
<td>0.8825</td>
</tr>
<tr>
<td>1 &amp; Up</td>
<td>0.1175</td>
</tr>
<tr>
<td>1</td>
<td>0.1103</td>
</tr>
<tr>
<td>2</td>
<td>0.0069</td>
</tr>
<tr>
<td>3</td>
<td>0.0003</td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8 &amp; up</td>
<td></td>
</tr>
</tbody>
</table>

Table 8 shows that the probability of finding a herd in an area is much higher if the average number of herds per area is 1 or 2 herds as compared to \( \frac{1}{8} \) to \( \frac{1}{4} \) herds per area. As stated above, the average number of herds per area for any county is to be one herd per area. With areas of this size, herds coming from the more dense areas of the county are more apt to be selected.
Known probabilities rule is obeyed: The conclusion from Table 8 is that the rule that every herd in the county have a known probability unequal to zero of being selected is observed on a practical basis when areas of a county equal to an average of one herd per area for the entire county are used as the basis of selection. This results from probabilities of selection which have a close relationship to the varying density of herds in the county.

Weld County, Colorado, is an example of a county where the density of herds can be quite high in parts of the county and quite low in other parts of the county. This can occur in states such as Iowa or Ohio which have a lot more uniform agriculture than the states of the western United States.

The use of townships and sections will work very well for states such as Ohio. Tennessee is a problem. In the case of Tennessee, coordinate lines could be drawn on appropriate maps. In any given county in any given state, there will be many areas without herd. The other areas will have one or more herds.

Known probabilities rule is not obeyed: There is no rational way to compute the probability of selection with the use of the random walk or drive. This is due to an unknown number of acres of farm land being represented by each mile of roadway and, hence, the probability of selecting any given herd of the needed size is unknown and varying and not necessarily related to the varying density of livestock in the county.

PAST SURVEYS IN VETERINARY SERVICES

The author had the problem of estimating disease rates soon after joining the Biometrical Services Staff (BSS) of ARS 22 years ago. Results of two surveys in the Animal Health Division (ANH), a predecessor of VS, are relevant. One survey was for trichinae in garbage fed swine (Jefferies et al., 1966) while the other was for salmonella in basic feed mills (Allred et al., 1967).

In each survey, the basic sample unit was not the herd or feed mill selected at random from the population at large. Instead, the basic sampling unit was the individual animal within the herd for the trichinae survey and the individual feed sample from the feed mill for the salmonella survey.

Population unit of interest: The nature of the estimation problem for the NADDS system is identical in that a herd selected at random is not the unit of interest. Instead, the individual animal in the herd is the unit of interest. This contrasts with SRS inventory estimation where there is a value observed for a herd which is the number of animals in the herd.

Estimates of disease in animals are the estimates needed. However, since clusters of animals in a herd are the units sampled, variation among herds is important to the estimation problem. This means that the amount of variation from herd to herd that might be expected must be considered. In examining this problem, one can consider some results from the trichinae and salmonella surveys. Variance and percent positive estimates were
computed using cluster sampling formulae for proportions from Cochran (1963).

**Defined vs undefined populations:** The contrast in computing estimates for the trichinae and salmonella surveys shows the importance of a well defined population to sample from. Therefore, distributions supplied by SRS for the pilot projects are of great value. In the trichinae survey, a list of garbage feeders with the number of animals in each herd was available for the state. This permitted including the number of swine in each herd and for each state for weighting in the estimation process.

In contrast, for the salmonella survey, while a list of feed mills and an estimate of the finished feed production was available for each state, the production of total feed and each feed category for the individual mills was unknown. In addition, the production of each feed category by state was not known. This meant that feed category production by state or by feed mill could not be used in weighting in the estimation process and that only total finished feed production by state could be used for weighting.

**Survey results:** Table 9 has estimates of percent positive and the standard deviation. Both theoretical binomial and actual cluster variances were computed so as to have the increase in variances due to cluster sampling. 297 premises were sampled in the trichinosis survey out of several thousand in the United States. 724 feed mills were sampled in 26 states out of several thousand total basic feed mills. The average number of samples per mill varied from 2.10 for animal meal to 4.46 for cattle feed. Not all mills had all categories of feed.

It is noted that the estimate of percent positive for trichinae was 0.50 percent with a standard deviation of 0.13 percent. This was with 297 herds and 5,955 individual samples. This is a much lower rate than many of those that will be estimated in NADDS for animal diseases. More in line with what might be expected in estimating many of the diseases or disease conditions of interest in NADDS is the result for fish meal where the percent positive is 4.72 with a standard deviation of 0.92 percent. These confidence limits will be discussed later in relation to inferences about required sample sizes for NADDS.

<table>
<thead>
<tr>
<th>Type of Survey</th>
<th>Number of Clusters</th>
<th>Samples</th>
<th>Percent Positive</th>
<th>Std. Dev Binomial</th>
<th>Std. Dev Cluster</th>
<th>Ratio of Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichinosis</td>
<td>297</td>
<td>5,955</td>
<td>0.50</td>
<td>0.091</td>
<td>0.13</td>
<td>1.42</td>
</tr>
<tr>
<td>Grain</td>
<td>635</td>
<td>2,698</td>
<td>0.66</td>
<td>0.156</td>
<td>0.19</td>
<td>1.22</td>
</tr>
<tr>
<td>Oil Seed</td>
<td>621</td>
<td>2,629</td>
<td>2.28</td>
<td>0.291</td>
<td>0.32</td>
<td>1.10</td>
</tr>
<tr>
<td>Fish Meal</td>
<td>366</td>
<td>805</td>
<td>4.72</td>
<td>0.747</td>
<td>0.92</td>
<td>1.23</td>
</tr>
<tr>
<td>Animal Meal</td>
<td>414</td>
<td>869</td>
<td>31.07</td>
<td>1.570</td>
<td>2.18</td>
<td>1.39</td>
</tr>
<tr>
<td>Cattle Feed</td>
<td>582</td>
<td>2,597</td>
<td>0.85</td>
<td>0.180</td>
<td>0.22</td>
<td>1.22</td>
</tr>
<tr>
<td>Swine Feed</td>
<td>502</td>
<td>1,567</td>
<td>3.13</td>
<td>0.440</td>
<td>0.58</td>
<td>1.32</td>
</tr>
<tr>
<td>Poultry Feed</td>
<td>560</td>
<td>1,605</td>
<td>5.23</td>
<td>0.556</td>
<td>0.73</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Table 9. Comparison of Binomial and Actual Standard Deviations.
SURVEYS OUTSIDE OF VETERINARY SERVICES

Doane Agricultural Service Data

In further examining what might be expected in the case of NADDS sampling, disease incidence results from an animal health market study by the Doane Agricultural Service (1980) can be considered. It must be emphasized that the Doane data lacks estimates on monetary loss due to morbidity, has an unknown degree of diagnostic validity, and has little data on specific disease agents. This is why their data is inadequate for any of the potential users that desire this data including the U.S. Department of Agriculture.

Some data from the Doane study for 1979 are shown in Tables 10 and 11. It is noted that the data is reported as number of affected animals. This is the ultimate necessary way of obtaining NADDS estimates in order to have estimates of economic loss. Results are shown for certain classes of livestock and certain disease classifications. The denominator in Table 10 represents the January 1, 1980, inventory plus animals sold and purchased during 1979. This is the denominator that Doane uses in computing death loss rates.

Table 10. Data from Doane Agricultural Service Disease Incidence Package.

<table>
<thead>
<tr>
<th>Livestock Class</th>
<th>Inventory, Sales, and Purchases</th>
<th>Disease Class</th>
<th>Number Affected</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Cattle</td>
<td>22,879,671</td>
<td>Total Death Loss</td>
<td>1,514,647</td>
<td>6.62</td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>12,955,127</td>
<td>Total Death Loss</td>
<td>278,478</td>
<td>2.15</td>
</tr>
<tr>
<td>Beef Cattle</td>
<td>139,618,690</td>
<td>Total Death Loss</td>
<td>3,806,957</td>
<td>2.73</td>
</tr>
<tr>
<td>Swine</td>
<td>233,046,866</td>
<td>Total Death Loss</td>
<td>15,208,938</td>
<td>6.53</td>
</tr>
</tbody>
</table>

Table 11. Data from Doane Agricultural Service Disease Incidence Package.

<table>
<thead>
<tr>
<th>Livestock Class</th>
<th>Inventory</th>
<th>Disease Class</th>
<th># Affected</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Cattle</td>
<td>18,138,504</td>
<td>Disease Death Loss</td>
<td>1,232,624</td>
<td>6.80</td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>10,392,514</td>
<td>Intestinal Problems</td>
<td>1,595,955</td>
<td>8.80</td>
</tr>
<tr>
<td>Beef Cattle</td>
<td>75,538,626</td>
<td>Disease Death Loss</td>
<td>1,865,872</td>
<td>17.95</td>
</tr>
<tr>
<td>Swine</td>
<td>65,149,954</td>
<td>Swine Influenza</td>
<td>8,132,723</td>
<td>12.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TGE</td>
<td>4,834,059</td>
<td>7.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intestinal Problems</td>
<td>23,257,424</td>
<td>35.70</td>
</tr>
</tbody>
</table>
Nature of denominator: Since the author disagrees with this construction of the denominator, the denominator in Table 11 represents only the January 1, 1980, inventory and this denominator is used for purposes of illustration. In contrast, studies in Minnesota constructed the denominator from animal months at risk. This permitted the construction of an annual rate based upon the average number of animals at risk per month over a 12-month period.

Table II shows that, based upon clinical observations, 47,036 beef cattle were estimated to have anaplasmosis. In the random sample survey for anaplasmosis in 1973 using MCI brucellosis blood samples, it was estimated that there were 4,890,500 beef cows affected in the United States. Despite the unknown degree of comparability of clinical observations and serological results, the estimate shown in Table II for beef cattle casts doubt on the Doane incidence estimates as far as diagnosis is concerned.

Minnesota validation study

In further examining what might be expected from NADDS, results of a validation study of a practicing veterinarian (DVM) based animal disease reporting system developed in Minnesota (Diesch et al., 1981) can be considered. The basis of the Minnesota system was a sample of clients of a random sample of practicing DVM's. The purpose of the study by Diesch et al. was to examine the validity of the Minnesota system by comparing a sample of clients of a random sample of practicing DVM's and a random sample of the entire population.

Table 12 has selected data from the validation report. For most diseases, the results of the random sample of the entire population ranged from about equal to significantly less than the results of the random sample of clients. This finding caused Diesch et al. to recommend a regulatory VMO based system rather than a practicing DVM based system.

Table 12. Selected Data from the Minnesota Validation Study.

<table>
<thead>
<tr>
<th>Livestock Class</th>
<th>Disease Class</th>
<th>Validation Sample</th>
<th>Client Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate</td>
<td>Std. E.</td>
<td>Rate</td>
</tr>
<tr>
<td>Dairy Cows</td>
<td>Lice</td>
<td>9.0 %</td>
<td>5.4 %</td>
</tr>
<tr>
<td>&quot;</td>
<td>Abcesses</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>Misc. Mastitis</td>
<td>18.0</td>
<td>3.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>Total Mastitis</td>
<td>29.1</td>
<td>44.9</td>
</tr>
<tr>
<td>Dairy Heifers</td>
<td>Lice</td>
<td>16.3</td>
<td>9.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>Influenza</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Breeding Swine</td>
<td>TGE</td>
<td>32.1</td>
<td>21.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>Total Enteritis</td>
<td>32.2</td>
<td>12.6</td>
</tr>
<tr>
<td>Pigs to Finish</td>
<td>Influenza</td>
<td>13.3</td>
<td>7.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>E. Coli</td>
<td>18.9</td>
<td>6.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>TGE</td>
<td>5.8</td>
<td>4.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>Dysentery</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>Misc. Enteritis</td>
<td>18.8</td>
<td>6.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>Total Enteritis</td>
<td>44.4</td>
<td>132.6</td>
</tr>
</tbody>
</table>
There was one glaring exception. This was transmissible gastroenteritis (TGE) in breeding swine. The rate for TGE was 32.1 percent for the random sample of the entire population and only 1.5 percent for the sample of clients. Diesch (1985) reported that the random sample was affected by a bad TGE outbreak in one large swine herd. This shows the importance of stratifying by herd size.

**Difference in confidence limits:** In further considering results from Table 12, some disease conditions and diseases have narrower confidence limits in terms of percent of the point estimate than other disease conditions or diseases. For example, miscellaneous mastitis in dairy cows has much narrower confidence limits than TGE in either breeding swine or farrow-to-finish pigs.

**Comparison of results from Doane and Minnesota validation study:** In comparing data from Doane in Table 11 with data from Minnesota in Table 12, these two diseases are of interest. The rate for mastitis is 17.95 percent in Table 11 and 29.1 percent in Table 12. These two rates appear fairly similar considering the nature of the two sets of data including the fact that Minnesota represents only one state.

**TGE comparison:** A comparison of the results for TGE is more difficult. This is due to a lack of comparable data as there is data on total swine for the Doane report and on two classes of swine for the Minnesota study. If the inventory for December 1, 1979, for breeding and market class swine for the U.S. is used, one can apply the rates obtained in the Minnesota study. The inventory is 9,655,000 head for breeding swine and 57,699,000 head for market swine.

While it is actually nursing pigs that are affected by TGE, the rates in the Minnesota study are based upon the number of pigs from birth to finish. These rates are annualized on the basis of pig months at risk. Since there are about two pig crops per year and since market swine are sold at approximately six months of age, the denominator for these rates is approximately equal to the number of market swine on hand. Consequently, the number of market swine is a reasonable figure to use as the population value for expanding the estimated rate to the population at large.

The estimate of TGE affected animals would be 3,099,255 in breeding swine and 3,346,542 in market swine for a total of 6,445,797 animals affected with TGE in the U.S. based on the Minnesota data. This compares with the Doane estimate of 4,834,059 animals. Of course, this is not a valid comparison due to the limited scope of the Minnesota data. Also, the definition of breeding swine may be broader in the December 1 SRS inventory than in the Minnesota study.

**Inference:** The main inference from this comparison of results of the two sets of data, despite the small sample size in the Minnesota study with 30 dairy herds, 29 herds with breeding swine, and 38 herds with farrow-to-finish pigs, is that confidence limits from the Minnesota study are fairly
narrow and that the results for the two diseases examined are not too different.

**British Surveys**

Several animal disease surveys have been conducted in Great Britain. These surveys are summarized by Leech (1971a and 1971b) and by Leech and Sellers (1979) and provide some useful insights into the problems of conducting surveys for incidence and prevalence.

**INFERENCES ON REQUIRED SAMPLE SIZE**

A consideration of the confidence limits from the Minnesota study and those from the VS surveys on trichinae and salmonella brings up an important aspect of the animal disease estimation problem. This is the precision in terms of the width of confidence limits which is required. For example, the January and July 1983 SRS Cattle inventory reports state that the standard error of the national estimate for total cattle and calves is less than 1 percent for the January report and about 1 and 1/2 percent for the July report.

**Disease estimate accuracy:** Experience shows that much wider confidence limits as a percent of the estimate of total affected for any given disease or disease condition are acceptable for the national estimate. In addition, the cost of obtaining an individual herd is such that it is not cost-beneficial to obtain confidence limits anywhere as narrow as those needed in inventory estimation.

It is noted that for endemic diseases, average estimates for 2 to 4 years may be satisfactory. This means that a smaller number of herds would suffice for adequate estimates than when accurate estimates of trend are needed.

**Among herd variation:** It is obvious that the amount and nature of variation among herds is important in considering sample size in terms of number of herds needed. Conditions such as mastitis and calf scours occur in most herds and have different variance characteristics and require a smaller sample size in terms of number of herds than will diseases that are spread by animal movement.

In further considering the confidence limits that might be obtained and their relationship to required sample size for a national NADDS, one can consider preliminary results for NADDS from Ohio and Tennessee for the first several months, variation for brucellosis infected herds, and results from an anaplasmosis survey in Texas (Alderink, 1984).

**Sample size — confidence limits:** Confidence limits are reduced by about one-half when sample size is increased by four. The Minnesota validation study had samples of about 30 herds. If sample size had been about 250, confidence limits would have been reduced about two-thirds.

The conclusion is that a national sample of about 1,000 herds is adequate. This sample size should permit accurate estimates for regions of the United States. This is in line with the author's prior intuition based upon
the trichinae and feed mill surveys that a sample of 1,000 to 1,500 herds would be adequate. A further inference is that samples of 60 herds will provide sufficiently accurate estimates for a few diseases and that samples of 250 herds will provide sufficiently accurate estimates for most diseases.

SUMMARY

Problems in securing statistically valid information on animal disease prevalence and incidence have been discussed. The relationship of these problems to the development of a National Animal Disease Detection System (NADDS) which is intended to obtain valid estimates of incidence and prevalence have also been discussed.

REFERENCES

disease reporting. Unpubl. working paper. BSS, ARS, USDA, Done for Nat.
brucellosis eradication program. BSS, ARS, USDA. July 14, 1961: 2p.
19. Diesch, S. L., D. W. Johnson, F. B. Martin, L. T. Christensen, R. Rövsbech, and
T. Therrien. 1981. Validation of the Minnesota food animal disease reporting
system. Final Project Report of Coop. agreement between U. of Minn. and VS,
APHIS, USDA. VS, APHIS, USDA, Hyattsville, Md. 97p.
Minnesota disease reporting system for food producing animals. Proc. 87th
Service, St. Louis, Missouri, August 1, 1980.
23. Harvey, W. R. 1958. Problem concerning the number of animals to test for
brucellosis in range areas. BSS, ARS, USDA. Sept. 30, 1958. 4p.
349-357.
25. Leech, F. B. 1971a. A critique of the Methods and Results of the British
National Surveys of Disease in Farm Animals, I. Discussion of the Surveys.
26. Leech, F. B. 1971b. A critique of the Methods and Results of the British
National Surveys of Disease in Farm Animals, II. Some General Remarks on
Assn. 350–358.
Unpubl. working paper. VS, APHIS, USDA. June 17, 1980. 8p.
32. Snedecor, G. W. 1947. An experiment in the Collection of Morbidity and
Assn. 218–225.
REPORT OF THE COMMITTEE ON ANIMAL DISEASE SURVEILLANCE

November 1, 1985

Chairman: Dr. G. C. Poppensiek, Ithaca, NY

J. A. Acree, CA; F. J. Alderink, MD; R. K. Anderson, MN; V. C. Beal, Jr., MD; Charles W. Beard, GA; Douglas L. Berndt, MD; S. L. Diesch, MN; C. R. Dorn, OH; J. G. Flint, MN; James W. Glosser, DC; Harry E. Goldstein, OH; Harvey S. Goss, GA; R. F. Hall, GA; C. M. Hibbs, NM; David W. Hird, CA; M. E. Hugh-Jones, LA; Larry Hutchinson, PA; Norman E. Hutton, OR; J. L. Hyde, MD; Robert F. Kahrs, MO; Lonnie J. King, MD; Herbert Lloyd, FL; L. D. Mark, VA; Frank B. Martin, MN; Hunt McCauley, MT; H. A. McDaniel, MD; David J. Meisinger, IA; W. R. Miller, MD; L. G. Morehouse, MO; R. S. Morris, MN; T. G. Murnane, TX; J. C. New, TN; S. R. Nusbaum, NJ; Joseph C. Paige, MD; P. A. Pickerill, IA; E. I. Pilchard, MD; J. C. Prucha, MD; Philip Ross, DC; Leon H. Russell, TX; Vaughn A. Seaton, IA; G. H. Snoeyenbos, MA; C. D. Van Houweling, VA.

The meeting of the Committee on Animal Disease Surveillance was held on Thursday afternoon, October 31, 1985 in the Marc Plaza Hotel, Milwaukee, Wisconsin. Fifteen committee members and more than thirty guests were in attendance.

Dr. Phyllis York, staff veterinarian for APHIS, USDA, Hyattsville, MD, reviewed the status of the National Animal Disease Detection System, addressing the current status of activities, citing projects in process in six states and plans to implement projects in two additional states. Extramural collaboration has been formalized with eight universities and with the Communicable Disease Centers in Atlanta, GA. NADDS has moved beyond the limits of a pilot program in the six initial states to a transitional stage in which cooperating states are assuming a greater lead role.

As one would anticipate for any innovative program, there are growing pains. For NADDS these include two of noteworthy concern:

1. [*There is the challenge of confidentiality; the challenge of assuring cooperating farmers and ranchers anonymity, so that findings of disease problems on a given farm or ranch will not jeopardize the livelihood of the producer, and at the same time recognize the responsibilities of disease control agencies of government.

The Committee commends those officials who are participating in the dialogue, and who are probing and assessing the ethical and legal concerns, with full confidence that controversial issues can be resolved intelligently and wisely.]

Committee members expressed concern about the confidentiality clause as

*Amended by the executive committee of USAHA.
it is inconsistent with current legal, scientific and ethical standards. As it is a basic aspect of the program it is essential that the matter be clarified to avoid confusion, and waste of time, money and effort.

2. Another growing pain is management of data; getting proper statistical inference so that conclusions from analysis of data are valid. Expedience in process is imperative. APHIS is urged to give this strong administrative priority.

On-going evaluation of the process to assure statistical validity is an active part of the developing program.

Dr. Rebecca Schiller of the Communicable Disease Centers in Atlanta, Georgia, addressed the Use of NADDS to Monitor Human Health Hazards. She complimented the planners of the NADDS system for the focus on statistical validity. Random selection supports the use of inferential statistics in assessing incidence, prevalence, and patterns of disease movement. Animals often are sentinels of environmental intoxications, which also threaten humans. Environmental exposures usually are to low levels over a long and frequently intermittent time period.

Primary or subset sampling by NADDS can be enormously useful in identifying and monitoring such environmental intoxications.

Canada and our Latin American neighboring countries are involved in national interests in animal disease surveillance.

Dr. Hector Campos Lopez, Director of Animal Health Programs, Inter-American Institute for Cooperation on Agriculture, a unit of the Organization of American States, discussed surveys of Latin American countries to assess current reporting systems for diseases of food-producing animals and interest in expanding the resourcefulness of data management systems.

Dr. Jorge Vargas, Director of Animal Health, Ministry of Agriculture, Republic of Mexico, discussed Mexico's pilot program for food-animal disease surveillance, showing an approach quite parallel to that of APHIS but with slightly different protocol.

Dr. John A. Kellar presented a Progress Report Toward Formal Food-Animal Disease Surveillance in Canada, stressing the importance of proper sampling to assure valid statistical inference from the data input.

A copy of the agenda is appended hereto.

Special topics were addressed by Dr. Victor C. Beal, Jr. of APHIS, including estimation problems. Dr. Billy Heron of California discussed the evaluation of list frames and area frames for disease surveillance of California swine.

Dr. William Owens of the College of Veterinary Medicine, Iowa State University has incorporated the NADDS procedures in his teaching program; practical, dynamic effective epidemiology, with economic evaluation as the bottom line.

Dr. Lawrence G. Morehouse of Missouri, representing the American
Association of Veterinary Laboratory Diagnosticians, reviewed the history of interest in Animal Disease Surveillance within that group of professionals, showing the parallel objectives with those of the Committee on Animal Disease Surveillance, USAHA.

NADDS is growing.

The formal papers will be submitted as supportive documents to this report.
RECENT RESEARCH ON BOVINE VIRAL DIARRHEA VIRUS: 
RELATIONSHIP TO VACCINES AND VACCINATION

Steven R. Bolin, DVM, Ph.D.
National Animal Disease Center, USDA/ARS

INTRODUCTION

Bovine viral diarrhea (BVD) virus is ubiquitous in cattle. The virus causes mild to fatal enteric disease, respiratory disease, reproductive failure, and congenital defects. Economic loss from BVD virus infection is due to prolonged calving intervals and reduced calf numbers, transient decreases in milk production or rate of gain, stunted growth, and death loss. The disease, BVD, occurs in 3 forms: an acute and usually subclinical form, a rare and highly fatal mucosal disease form, and a rare chronic disease form characterized by wasting and eventual death.

The purpose of this report is to review recent research on BVD and relate research findings to BVD vaccination.

Acute BVD

Although acute BVD is seldom a clinically severe disease, BVD virus infection produces transient but substantial effects on immune function. Leukopenia accompanied by depression of lymphocyte and neutrophil function occurs within a short period of time after exposure of cattle to BVD virus.1,2 Depletion of immune functions by BVD virus weakens the host's defenses to other infectious agents. Supporting this possibility are reports of bacteremia, increased tissue concentration and distribution of infectious bovine rhinotracheitis virus, and increased severity of Pasteurella haemolytica induced pneumonia associated with acute BVD.1,3,4

Modified live BVD vaccine virus also has depleting effects on lymphocyte and neutrophil function.5 Thus, use of modified live BVD virus vaccines in stressed and recently congregated cattle might have detrimental effects on health by exacerbating existing disease processes.

Other recent research indicates that there are differences between BVD viruses in pneumopathogenicity.6 Cattle were inoculated with one of two BVD virus isolates and then inoculated several days later with P. haemolytica. There were significant differences in the severity of respiratory disease associated with the viruses, indicating differences between BVD viruses in potentiating bacterial pneumonia. This research involved field isolates of virus. Would similar results have been obtained with two vaccine viruses? Would vaccine A be safer than vaccine B by having less synergistic effect on post vaccinal respiratory disease while vaccine B might have less synergistic effect for enteric disease?

The above research has established that BVD viruses, wild type or vaccine strains, affect immune function and some researchers are exploring potential synergism between BVD viruses and other infectious agents. Purposefully or not, this research raises the question of safety of
modified live BVD virus vaccines. Is it possible that vaccination at the “wrong” time is potentially more harmful to health than not vaccinating?

**Mucosal Disease and Chronic BVD**

Researchers recently demonstrated that mucosal disease and chronic BVD are sequelae to congenital persistent infection with noncytopathic BVD virus. Persistent infection is established through fetal exposure with virus before day 125 of gestation. Mucosal disease and chronic BVD occur after persistently infected cattle become superinfected with cytopathic BVD virus. The modified live BVD virus vaccines contain cytopathic BVD virus. Thus, it is possible that vaccination of persistently infected cattle incites rather than protects against disease.

A disease similar to mucosal disease occurs infrequently after vaccination for BVD. Both noncytopathic and cytopathic BVD viruses have been isolated from spleens of cattle with fatal postvaccinal mucosal disease. However, attempts to experimentally produce mucosal disease in persistently infected cattle by vaccination were unsuccessful. Although vaccination failed to induce mucosal disease, several unexpected and important findings were made during the study. A substantial and specific neutralizing antibody response was detected to the vaccine virus and other presumably related BVD viruses, indicating persistently infected cattle are not immunotolerant to BVD virus. Neutralizing antibodies were seldom detected to the persistent noncytopathic virus or to a few other BVD viruses. Also, vaccination did not protect the persistently infected cattle from developing fatal mucosal disease after challenge exposure with a second cytopathic BVD virus.

The above findings might indicate mucosal disease requires specific combinations of noncytopathic and cytopathic BVD viruses. Thus, vaccination of cattle persistently infected with a certain noncytopathic BVD virus might not induce mucosal disease but the same vaccine given to a cow persistently infected with a different noncytopathic virus would result in mucosal disease. If this hypothesis is correct, mucosal disease death losses directly attributable to vaccination would be some fraction of the number of persistently infected cattle vaccinated each year. Persistent BVD virus infection in cattle has a clustered distribution with a frequency that might be as high as 1% in weanling calves. The expected yearly incidence of postvaccinal mucosal disease would be low, with very few herds suffering most of the losses. This is what has occurred since modified live BVD virus vaccines became available.

Assuming that research eventually proves BVD vaccination causes mucosal disease, who is liable for losses attributable to vaccination? How are we to determine which cattle are persistently infected before vaccination? Will timely vaccination of the dam prevent in utero BVD virus infection of the fetus, reducing or eliminating persistent infections? Who is liable for persistent BVD virus infection resulting from adventitious noncytopathic BVD virus in vaccines given to pregnant cattle? Will calves vaccinated, purposefully or not, with noncytopathic BVD virus and then
put back with their pregnant dams transmit the virus to the dam and her fetus?

SUMMARY

Current vaccines for BVD are effective for preventing acute BVD in cattle. However, recent research indicates untimely vaccination for BVD may enhance detrimental effects on health by other infectious agents. Under certain conditions, BVD vaccination may even induce fatal disease. Questions concerning BVD vaccination are related to the ability of killed or live virus vaccines to protect against BVD and persistent BVD virus infection, while not enhancing other diseases or causing persistent BVD virus infection.

REFERENCES


REPORT OF THE COMMITTEE ON BIOLOGICS

Chairman: R. W. Loan, College Station, TX
Vice-Chairman: Majon Huff, Denver, CO

J. Bruce Addison, MO; D. C. Alexander, Canada; Miles H. Bairey, IA; W. H. Beckenhauer, NE; Neal Black, MN; Mary Lou Chapek, NE; M. L. Crandall, MD; David A. Espeseth, MD; John Finnell, IL; Joe S. Gloyd, IL; James A. Gourlay, CA; R. E. Hall, WI; B. B. Hancock, IA; Wade L. Kadel, KY; D. E. Kahn, NJ; Robert F. Kahrs, MO; M. J. Kemen, NY; L. H. Lauerman, Jr., AL; Vincent Marshall, NE; John P. Newman, MI; Duane Pankratz, SD; R. J. Price, MD; D. C. Randall, Jr., IA; R. C. Stewart, KS; O. H. Timm, CA; J. D. Todd, KS; P. R. Turner, TX; H. E. Vanderslice, DE; Marty Vanier, VA; R. D. Welsh, TX; G. B. E. West, CA; Robert D. Williams, IN; J. M. Williams, MO; W. H. Wohler, TX.

Thirty-four committee members and other interested persons attended the Biologics Committee meeting on Tuesday, October 29, 1985. The Chairman distributed copies of the Melcher Amendment to the Farm Bill which amends the Virus-Serum-Toxin Act of 1913. Additional related information was also distributed. Adoption by USAHA of the Committee's 1984 resolution in support of biologics regulation by USDA was reviewed.

It was announced that persons wishing to be appointed to the Biologics Committee should contact the Chairman. Appointments will be recommended with regard to balance in committee membership between industry, producer and government groups.

Dr. Joseph M. Cummins, Texas Agricultural Experiment Station, Amarillo, Texas, reported on the adjuvant effect of human interferon alpha on the immune response of cattle. Human interferon alpha is licensed by the Department of Health in Texas for use in cattle. This interferon was tested in cattle given bovine viral diarrhea (BVD), parainfluenza 3 (PI3), and/or infectious bovine rhinotracheitis (IBR) virus vaccines. Calves given interferon and vaccine responded with more seroconversions and higher geometric mean titers of antibody than calves given vaccine alone. This enhanced response was seen only with modified live viral vaccines.

Dr. Steven R. Bolin, Agricultural Research Service, Ames, Iowa, reported on the immunology of BVD and the effect of vaccination on other infections. Current vaccines for BVD are effective for preventing acute BVD in cattle. However, recent research indicates untimely vaccination for BVD may increase the severity of other infections. Under certain conditions, BVD vaccination may induce fatal disease.

Dr. Charles W. Purdy, USDA, Bushland, Texas, reported on a survey of vaccination practices in five southeastern states. The use of vaccines increased with increasing herd size. Older producers vaccinated less than younger producers. Seventy-five percent of the producers vaccinated their own animals and 62.5% of owners rarely or never used a veterinarian. Eighty-six percent of the calves included in the survey were vaccinated for...
blackleg. Dr. Purdy reported that the widespread practice of vaccinating for blackleg appeared to afford an opportunity for initial vaccination against bovine respiratory disease.

Dr. David Espeseth discussed organizational restructuring and program activity in Veterinary Biologics. Presently there are 65 licensed establishments and 1,290 licensed products. Consideration is being given to minor species products and conditional licenses. The Department is considering minor species label claims and making funds available to the IR-4 program. Future plans include reduced regulation where this is feasible. Dr. Espeseth stated that the Department supports the amendment to the Farm Bill (Melcher Amendment) calling for regulation of veterinary biologics not now covered under present laws.

Mr. John Thomas, attorney for the Animal Health Institute, commented on the status of the Farm Bill which contains provisions which amend the Virus-Serum-Toxic Act.

A discussion of the Melcher Amendment to the Farm Bill which amends the Virus-Serum-Toxin Act of 1913 followed. The amendment would:

1. Consolidate in USDA jurisdiction over all animal biological products regardless of whether they are transported across state lines or not.

2. Exempt animal owners and veterinarians from licensing requirements for biologics prepared for use in their own animals (owners) or for use in animals under their professional care (veterinarians).

3. Exempt from licensing requirements “intrastate” animal biologics produced and marketed in states with qualified state programs certified by USDA for intrastate biologics regulation.

4. Provide for special licenses under expedited procedures to meet emergency conditions, limited market needs, local needs, or other special circumstances.

5. Provide for a 4-year phase in period with a possible 5th year extension.

6. Provide enforcement provisions with civil remedies of detention, seizure, condemnation, and injunction.

A resolution was passed for presentation to the Executive Committee.
BLUETONGUE VIRUS INFECTION OF CATTLE: CURRENT STATUS AND RESEARCH TRENDS

J. L. Stott¹ and B. I. Osburn²

From the Departments of Microbiology and Immunology¹ and Pathology², School of Veterinary Medicine, University of California, Davis, CA 95616

Bluetongue virus (BTV) infection, and disease, in both domestic and wild ruminant species continues to be of major importance at both the national and international level. The intent of this paper is to briefly review the current knowledge and areas of active research on BTV infection relative to cattle. The aspects to be discussed will include virus characterization, pathogenesis, epidemiology and diagnostics.

BTV is the prototype orbivirus in the Reoviridae Family. Twenty four serotypes have been recognized worldwide¹ with five being present in the U.S. While serotypes 10, 11, 13 and 17 have been recognized within the U.S. for many years, serotype 2 was first identified in a sentinel cattle herd in Florida in 1982.²³ Since that time the virus has been isolated from several regions in Florida⁴ and more recently associated with clinical disease and abortion in Alabama.⁵ Thus it appears inevitable that serotype 2 will soon be introduced into many areas of the U.S. and become endemic. BTV serotypes are classified by in vitro virus neutralization. These serotypes have limited and varied cross-protecting activity in sheep.

BTV infection of cattle is common in much of the U.S. with its distribution being largely dictated by vector activity. The primary vector(s) of BTV appear to be Culicoides species; in the U.S., C. variipennis has been incriminated as the major vector.⁶⁷ A recent report has also demonstrated experimental transmission of BTV by the argasid tick, Ornithodoros coriaceus.⁸ These ticks were infected by feeding on cell cultures and chicken embryos infected with serotype 13, with subsequent transmission to a cow. The report suggests the tick may serve as a potential biological vector and reservoir of BTV in the western U.S. However, its role in the epidemiology of BT is yet to be determined. BTV may also be transmitted via infected semen (bull to cow)⁹⁻¹¹ and from an infected cow to the developing fetus.¹²⁻¹⁵ The birth of viremic calves has been documented⁹,¹⁵ and some reports suggest these animals may serve as virus reservoirs for many years.¹⁶

While BTV infection of cattle is common, expression of overt clinical disease is relatively rare. BT disease in cattle has often been referred to as muzzle disease, mycotic stomatitis, ulcerative stomatitis and pseudo foot-and-mouth disease. Clinical expression of BT disease in cattle is variable and may include a transient febrile response, excessive salivation, dermatitis, localized inflammation with necrosis of the bucal mucosa, skin lesions on the udder, excoriation of the epidermis in the interdigital space, stiffness of gait, coronitis, lameness and loss of condition.¹⁷⁻²⁴ BTV pathogenesis in cattle is poorly understood and the factor(s) that dictate whether
disease will be expressed upon infection are speculative. Metcalf et al suggested that the lesions associated with clinical BT disease in cattle were the result of a hypersensitivity reaction induced by prior exposure to other serotypes of BTV or related orbiviruses. Subsequent to this speculation, experimental reproduction of a clinical syndrome in cattle was reported that closely resembled the natural disease. Cattle were given two inoculations of inactivated BTV in adjuvant in association with simultaneous administrations of the immunomodulators, cimetidine or levamisol. Upon live virulent virus challenge, these animals developed clinical disease. The basis of this disease process was speculated to be due to a Type I hypersensitivity based upon identification of virus-specific IgE in sensitized cattle, elevation in plasma levels of histamine and prostaglandin E2, and elevated tissue levels of histamine. In support of this experimental study, a natural case of BT disease in a heifer has recently been reported with similar findings. Thus it would appear that expression of BT disease in cattle is dependent upon prior sensitization by antigenically related virus(es).

BTV infection of pregnant cattle can result in fetal disease. The sequel to BTV infection of the fetus is dependent upon fetal age and the infecting virus. Bovine embryos with an intact zona pellucida are resistant to infection in vitro; subsequent to hatching, virus infection results in embryonic death. Experimental studies employing direct inoculation of the developing fetus during mid-gestation (85–125 days gestation) with serotype 11 results in fetal death whereas serotypes 10, 13 and 17 caused hydranencephaly with fetal development continuing to term or near term. In the latter case, all fetuses developed an immune response and cleared the virus by term. These studies were in contrast to those reported by Luedke et al in which calves were born with a multitude of anatomical anomalies, were persistently infected, and immunologically unresponsive to the virus. Recent studies conducted using three distinct genotypes of serotype 11 in a mouse model have demonstrated variable virulence for suckling mice, dependent upon genotype, i.e. two strains were virulent and 1 was avirulent. The recognition of such strain-dependent virulence within a given serotype provides one potential reason for the contrasting studies in the developing bovine fetus. The strain, or genotype, of virus used may well dictate the outcome of fetal infection.

The epidemiology of BTV is complex and much remains unknown as to the basis of serotype and strain evolution, virus reservoirs, and means of transmission and their significance in nature. Extensive epidemiologic studies in the western U.S., primarily California, have demonstrated BTV infection and disease to be seasonal in nature. This activity, which is typical of July through October, is consistent with vector activity and dictated by seasonal temperatures. Thus mild weather in late spring or early winter may permit some virus activity. Multiple serotypes of virus are commonly active during a given year in California (serotype 10, 11, 13 and 17). Such multiple serotype activity is also commonly observed on
individual farms and in individual animals. Up to three distinct serotypes have been isolated from animals on a single premise on a given day and up to two distinct serotypes have been isolated from a single blood sample obtained from an individual bovine. In addition to the multiplicity of BTV serotypes, recent studies have identified multiple genotypes within a serotype, as determined by gel electrophoresis of viral genomic RNA. A heterogenous population of genotypes representing multiple serotypes are commonly active within given geographic areas, specific herds, and individual animals. The significance of this genetic diversity, relative to serotype evolution, protective immunity, virulence and virus persistence is unknown. In the case of a sentinel study, animals infected with multiple genotypes and serotypes were viremic for a longer period of time relative to those animals from which only a single virus was isolated. While limited in scope, this study would suggest that the presence of multiple viruses within a given animal may well contribute to prolonged viremias.

Diagnosis of BTV infection has become increasingly important due to restrictions placed upon serologically positive animals for international export. In addition, diagnosis is also required for confirmation of clinical disease, confirmation of fetal infection, and epidemiological studies directed at defining BTV activity. Diagnosis of BTV infection has typically relied upon clinical observation, pathological lesions, serology and virus isolation. Clinical BT disease in cattle is often misdiagnosed due to its similarity to other vesicular diseases. The only histological descriptions of clinical BT in cattle are those describing biopsies of skin lesions obtained from infected animals; such lesions were inflammatory in nature and characterized by perivascular infiltration of mononuclear cells and eosinophils, and edema. Pathologic diagnosis is not typical since infected cattle, including those exhibiting overt clinical disease, experience very low mortality.

The use of serology in diagnosis of BTV infection is customary. Various immunologic assays for BTV have been described in the literature that detect serotype specific or group specific antibodies; the agar gel immunodiffusion (AGID) assay is the most universally used test. While serology is an invaluable tool for identifying past exposure on a herd basis, it has often been inappropriately used to retrospectively associate BTV with disease and/or abortion, and label positive cattle and their genes (semen and embryos) as unsafe for export. Epidemiologic studies have demonstrated the seasonal nature of virus activity. In excess of 3,000 cattle in California were tested for BTV and specific antibodies over a three year period during the months of January through June; 33% were seropositive and only one virus isolation was made (isolated in June). This information was evidence that during the cold winter months seropositive cattle were not viremic. Additional concern over the use of serology (AGID) for identifying suspect virus carriers has been the isolation of virus from seronegative cattle. In a recent study, 40% of field infected cattle (82 of 206 viremic animals) were seronegative at the time of virus
isolation. This data, in addition to the report of experimental persistently infected and immunologically unresponsive cattle, caused extensive concern, especially in relation to export. A sentinel study employing 10 calves produced similar data; no animals were seropositive at the time of the first isolation. However, two weeks later, all animals seroconverted. Thus in the case of the epidemiologic study, it is probable that the 40% virus positive/AGID negative animals were merely bled early in the infection and if follow-up bleedings had been possible then seroconversion would have been detected. The persistence of antibodies following a BTV infection is variable and probably affected by virus strain, immunologic responsiveness of the individual animal and the number of exposures to virus. Two of ten animals in the sentinel study previously mentioned became AGID negative one month after the last virus isolation.

Isolation of BTV is the ideal diagnostic choice. However, it must be appreciated that isolation is time consuming, expensive and often hindered by: a) poor specimen sample, b) virus strains that are difficult to adapt to culture systems (cell cultures and embryonating chicken eggs), c) interfering neutralizing antibody, or d) lack of virus titer due to its recent clearance. The latter case is best illustrated by the inability to isolate BTV from many newborn calves, still births or late term abortions induced by BTV infection at mid-gestation. While the virus was responsible for the pathology, it had been cleared by the time of birth. In such cases, serology is the most appropriate tool for diagnosing the etiologic agent. Cattle exhibiting acute clinical BT disease also often present a diagnostic dilemma. Cattle often develop obvious clinical signs of BT at a time when the viremia has been cleared or reduced to very low titer. In endemic areas the use of serology is also of limited value since the animal may have had an antibody titer induced by previous exposure. If in fact BT disease is a Type I hypersensitivity, then identification of BTV-specific reaginic antibody (IgE) by ELISA or passive cutaneous anaphylaxis (PCA) may prove to be of diagnostic value. In addition, skin biopsies taken from affected areas may prove to be of use in histopathologic preparations demonstrating perivascular inflammation with an eosinophilic component.

In recent years extensive research has been devoted to improve technologies for diagnosis of BTV infection. The most universally accepted technique for isolation is currently intravenous inoculation of 11-day-old embryonating chicken eggs (ECE's) followed by incubation at 33.5 C. This process of virus isolation is time consuming and expensive as multiple ECE and cell culture passages are often required to adapt the virus such that it can be identified as BTV and serotyped. Numerous studies have attempted to isolate virus on multiple cell lines in vitro. These studies have reported some success but the ECE still appears more effective on the majority of isolations.

Techniques for improving BTV diagnosis include the application of monoclonal antibodies, cloned cDNA probes, and western immunoblotting. Monoclonal antibodies have been successfully applied to the
identification of BTV in cell cultures by fluorescent antibody (FA) staining and in paraffin-embedded thin sections of infected ECE's by a peroxidase-anti-peroxidase (PAP) staining procedure. While these monoclonal antibody probes have yet to be tested on infected bovine tissues, the latter procedure holds promise as a useful diagnostic tool for identifying BTV proteins in necropsy tissues and blood cells.

Extensive effort has been directed at cloning a number of the genomic RNA segments of BTV for potential use as diagnostic reagents and recombinant vaccines. Cloned cDNA probes, representing several BTV genome segments, have been developed for use in northern blotting, dot-blotting and in in situ hybridization; cDNA probes have been developed that are serotype-specific and group-specific. These probes have been used to successfully identify the presence of BTV RNA in extracts of infected cell cultures and ECE's by use of northern and dot blots. Furthermore, these hybridization techniques have successfully employed biotin labeled cDNA probes that can be visualized following addition of avidin-enzyme (alkaline phosphatase or horseradish peroxidase) substrate systems; specific binding (hybridization) of the cDNA probe can then be visualized by enzyme-induced colored precipitates thus negating the use of radioisotopes. In situ hybridization techniques using enzyme- and radioisotope-labeled cDNA probes have successfully identified BTV in infected cell cultures and in blood cells obtained from infected sheep. These techniques hold considerable promise as potential tools for diagnosis of BTV infected cattle. However, considerable research remains to be conducted in order to determine relative sensitivity and specificity under various conditions. These techniques must be tested for ability to recognize multiple strains of BTV and must be successfully employed by independent laboratories. Further testing is needed to compare these diagnostic probes to existing diagnostic tests, i.e. embryonating chicken eggs.

While the use of recombinant technology in development of vaccines and diagnostic reagents is currently a novel and in-vogue area of research, studies directed at alternate methodologies and approaches for advancement of such research areas is necessary. Furthermore, basic research directed at defining BTV pathogenesis and the molecular basis of protective immunity will probably be required prior to the rational development of recombinant and/or synthetic peptide vaccines. Studies are currently being conducted to provide such needed information. The use of western immunoblotting is currently being used to study BTV protein-specific immune responses in ruminant species. These studies are expected to provide information on the viral protein(s) that elicit protective immunity; previous studies have provided evidence that virus neutralizing antibodies do not necessarily associate with protective immunity. Relative to improvement of diagnostics, the western immunoblotting procedure has also identified BTV protein-specific antibody responses that are transient and when present associate with a recent viral infection.
Such an assay may prove valuable in permitting exportation of serologically positive cattle or their semen and embryos.

In summary, advances are being made in BTV research relative to: a) defining the molecular basis of virulence and protective immunity, b) applying recombinant, monoclonal antibody, and immunoblotting technologies to advancing the area of diagnostics and c) better defining the molecular biology of BTV and related orbiviruses.

LITERATURE CITED


49. Cherrington, J. M., Ghalib, H. W., Davis, W. C., Osburn, B. I. 1984. Monoclonal antibodies raised against bluetongue virus detect viral antigen in


The Bluetongue and Bovine Leukosis Committee met at 1:30 p.m. on Wednesday, October 30, 1985. There were 20 members and 30 guests, a total of 50 people in attendance.

The Committee met as requested by the president of the USAHA to consider the business of the committee and submit the following report.

BLUETONGUE

Dr. Osburn conducted the discussion on bluetongue.

Dr. R. Sellers, currently with Agriculture Canada, gave an epidemiological assessment of bluetongue virus (BTV) infection on worldwide basis. The importance of epizootic hemorrhagic disease virus (EHDV) was also stressed. The distribution of these two viruses appears to be dependent on the presence of Culicoides species capable of transmitting the virus. Dr. Sellers described ecosystems of BTV infection throughout the world and included the Eastern Mediterranean and Gulf area, the Caribbean region, and the U.S. A serotype present in one region or ecosystem may be harmless, but if introduced into another region may become virulent. Six climatic zones, A through F were described and included: equatorial (A), tropical (B), Mediterranean (C), temperate (D), long winters (E), and long cold winters (F). BTV and EHDV are endemic in zones A–C, epidemic in zone D, sporadic in zone E and zone F is free of virus. Serological conversion to BTV occurs year-round in zone A, twice a year in zone B, once a year in June through December in zone C, every 2–7 years in zone D and every 20 years in zone E. Overwintering of the virus within a zone is dependent upon persistence of virus in the animal population, transplacental transmission, and in the insect vector. Virus spreads outside of a zone by animal movement or migration and infected insects being carried on the wind. Dr. Sellers discussed the practical problems associated with movement of animals and germ plasm throughout the various zones. Movement of
embryos can safely be done if the embryo is properly washed. Semen should be considered safe if the donor animal is virus free.

Dr. A. Luedke gave an update on research at the Arthropod-Borne Animal Disease Research Laboratory in Laramie. Studies are being conducted on BTV carrier cattle infected with plaque-cloned BTV. Carrier animals are sporadically viremic and immunologically unresponsive. Embryo transfer experiments are planned to be conducted in the future from persistently infected cows. Collaborative studies on development of diagnostic DNA probes are being conducted with Drs. Collison and Roy. Additional research at ABADRL includes development of monoclonal antibody-based ELISA systems, improvement of techniques for virus purification, biochemical characterization of the virus, field studies, and vector competence studies. Primary colonies of Culicoides obtained from New York were resistant to BTV infection but five generations later they became susceptible.

Dr. D. King, USDA/ARS, discussed the relocation of the Arthropod Borne Animal Disease Research Laboratory from Denver, Colorado to Laramie, Wyoming. Six locations were considered and Laramie was decided upon based on the presence of adequate space and relative expense. The program will emphasize research on improving diagnostic technology for facilitating export of animals and their germ plasm to foreign markets. A discussion followed Dr. King's presentation and Olin Timm stated that the research on vaccine development at ABADRL will be dropped.

Dr. C. Gipson, USDA, APHIS reported on the bluetongue programs within APHIS. The proposed conditions for potential international export of cattle from the U.S. was described and involved the designation of low, medium, and high risk geographic areas within the U.S., the conditions for export to Canada were described with the varying conditions being dependent upon the designated risk areas. Conditions under which U.S. livestock and germ plasm can be exported are dictated by the importing country and are often varied.

Dr. K. Squire reported on efforts to develop cDNA probes for identification of BTV in infected cells. The report represented research being conducted at the University of California at Davis. cDNA probes have been developed that are both BTV group reactive and BTV serotype specific. Probes have successfully been developed using either radioisotopes or enzymatic labels. Dot-blot assays have been developed for identification of virus RNA in extracts of infected cell cultures and embryonating chicken eggs. Techniques for in situ hybridization on tissues are currently being refined.

Dr. J. Stott reported on additional BTV research being conducted by scientists at the University of California at Davis. Studies are being conducted to develop new improved sensitive serologic and virologic techniques for diagnosing BTV infection. Western immunoblotting procedures have been developed that detect low levels of viral proteins in extracts of infected cell cultures. Attempts are also being made to identify viral
protein-specific antibody responses that would differentiate between infected or recently infected animals and those with long post exposure, studies are also being conducted to identify the molecular basis of BTV virulence, determine the basis of protective immunity, and determine the presence of BTV strain heterogeneity and its significance in BTV epidemiology.

Dr. A. Torres, Cornell University, Ithaca, New York, presented the Bluetongue program recently implemented in the State of New York. The program is designed to qualify New York cattle as being BTV-free. The program involves test and removal procedures on a voluntary basis with animal removal being dictated by management procedures. Testing will be done in the vector free season. Two consecutive negative serologic testings will be required to determine absence of virus from a given herd. A comprehensive BTV survey including animal exposure and vector competence is being planned.

Dr. W. Bulmer from Agriculture Canada reviewed Canada's BTV surveillance program. In 1976, 13 of 3,267 cattle tested were positive for BTV exposure. In 1984-85, 1 out of 4,986 animals tested positive. These animals were in British Columbia in the Okanagan Valley. Canada is currently testing 10,000 cattle annually for presence of BTV antibodies.

Dr. J. Pearson reported on BTV testing at the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. Three BTV isolations were made in FY1985 and all were serotype 13. A BTV survey was conducted in November 1984 on 9810 brucellosis cattle identification samples from 18 northern states. Forty-eight samples were positive by immunodiffusion (AGID). Forty-six of these positive samples were tested for neutralizing antibodies to the 5 U.S. serotypes and all were negative; 20 were positive for EHDV serotype 2 and 1 was positive for EHDV serotype 1. A similar BTV survey is currently in progress involving 29 northern states.

In January through April of 1984, 1,197 cattle were exported to China. All animals were tested to be virus and antibody negative. The requirements for virus isolation attempts from U.S. cattle for semen export to China, Europe, and Great Britain were reviewed; China requires inoculation of blood into embryonated chicken eggs (ECE), Europe requires inoculation of semen into ECE and cell cultures; and Great Britain requires inoculation of blood and semen into sheep.

NVSL is currently cooperating with the Universities of California-Davis and Alabama-Birmingham to evaluate cDNA probes for the diagnosis of BTV. Research conducted at NVSL also demonstrated that freeze-thawing of inoculated ECE's was as effective as grinding the embryos in BTV isolation. Relative to the AGID test, Dr. Pearson reported 60 laboratories were approved to perform BT export testing. The proficiency test for 1985 is currently ready to send to 81 USDA approved laboratories. Last
year, 2,499 ml of BTV antigen was shipped and was enough to perform approximately 378,450 tests.

Dr. A. Luedke reported on the current status of BTV serotype 2 in the U.S. He reported that the Ona B strain of serotype 2 was isolated by Dr. L. Lauerman in the state of Alabama. Isolates were made from cattle with clinical disease in July of 1984 and March of 1985. Serotype 2 was also isolated from a near term aborted fetus in January 1985. The movement of BTV serotype 2 into Alabama is the first indication of its pathogenicity in cattle. Dr. Luedke stated that the highly variable serotype 2 genome with its unpredictable pathogenicity is likely to cause problems for livestock producers in the future. Dr. Luedke also reported that Drs. Gibbs and Greiner isolated Ona B strain of serotype 2 from cattle in Florida in 1983 and 1984; BTV serotype 2 was first isolated in the U.S. in Florida in 1982.

BOVINE LEUKOSIS

Dr. Lyle Miller conducted the discussion on bovine leukosis.

Dr. Chester Gipson, USDA-APHIS, Hyattsville, MD discussed the position of Veterinary Services (VS) and activities relating to leukosis. He stated that VS does not advocate a national leukosis control program. However, VS does endorse the program approved by the bluetongue and bovine leukosis committee which was published in the 1982 USAHA Proceedings and supports initiation of voluntary programs by individual states. To facilitate movement of cattle in international trade, Dr. Gipson presented three possible requirements that could be offered in negotiations with importing countries. They are:

1. An animal should originate from a herd declared free of leukosis after the herd has had three negative tests conducted at 60 to 90 day intervals.
2. If the animal originates from a herd not free of leukosis, it must pass two negative tests at 21 day intervals and must be negative at the time of export.
3. A health certificate issued by an accredited veterinarian certifying the health status of the animal and of the herd of origin.

Dr. Alfonso Torres, Cornell University, Ithaca, NY reported that the State of New York has initiated voluntary control programs for bluetongue, bovine leukosis and Johne's disease. The programs are funded by the State of New York through its Department of Agriculture and Markets and are operated by the Veterinary Diagnostic Laboratory at Cornell University. The programs have resulted from the combined efforts of livestock organizations, individual herd owners, the diagnostic laboratory and the NY Department of Agriculture and Markets. Encouragement by neighboring states and other groups was also helpful.

The leukosis program is based on testing by the agar gel immu-
nodiffusion test and subsequent removal of seropositive animals from the herd. Four basic steps in the program are:

1. Initial testing of all animals six months of age or older.
2. Decision by the herd owner to enter the program. An agreement requiring specific management practices is signed by the herd owner, the attending veterinarian and the State of New York personnel.
3. Further testing at four to six month intervals and removal of seropositive animals is required. All animals six months of age or older are to be tested.
4. Certification requirements will follow the Uniform Methods and Rules for the Establishment and Maintenance of Designated BLV-free Herds as published in the bluetongue and bovine leukemia committee report in the USAHA Proceedings (1982).

During general discussion, it was reported that the State of Wisconsin has a leukosis control program in the planning stages.

Dr. William S. Bulmer, Agriculture Canada, Ottawa, Canada reported that approximately 9% of Canadian dairy cattle or 40% of the dairy herds and 0.5% of the beef cattle or 10% of the beef herds are seropositive for bovine leukosis virus (BLV) antibodies. In general, the prevalence is higher in the central provinces. Proposals for two types of leukosis control programs have been developed but the cattle industry has not chosen to support a control program. In artificial insemination centers, a program was initiated in 1979 to gradually eliminate BLV seropositive bulls. Since 1983, all bulls in AI centers are seronegative.

Dr. James E. Pearson, USDA-APHIS-NVSL, Ames, IA reported that a bovine leukosis proficiency test was completed by the 47 laboratories approved to do export testing. The average number of samples missed was 1.1. Laboratories that missed more than four samples were unsatisfactory. Twenty-six laboratories did not miss any samples. All of the unsatisfactory laboratories successfully completed a subsequent proficiency test. The 1985 proficiency test is ready to be sent to the 66 approved laboratories.

There were 2,238 samples tested at NVSL for BLV antibody and 311 (13.9%) were positive.

Dr. Janice Miller, USDA-ARS-NADC, Ames, IA reviewed recent literature relating to transmission, control and comparative virology of BLV.

Dr. Ronald Schultz, School of Veterinary Medicine, University of Wisconsin, Madison, WI discussed previous and current work on BLV. He reviewed data on the prevalence of infection in Alabama, Kentucky, New York and Wisconsin. In dairy cattle, approximately 15%, 25%, 25% and 40% were seropositive in New York, Kentucky, Wisconsin and Alabama respectively. The prevalence among beef cattle was 20% in Alabama and 25% in Kentucky. Within herds there is no correlation between the number of seropositive animals and the number that develop tumorous leukosis (lymphosarcoma). Transmission of BLV may be vertical (in utero) in some cases but horizontal spread is more common. Transfer of cells
containing viral information in the provirus state is an important aspect of transmission. Biting insects can be vectors but are not a major means of horizontal spread. Studies to determine the relative infectivity of individual animals examined the effect of natural or experimental exposure, the presence of lymphocytosis, the duration of infection and the age of the animal. Results indicated that the most infectious animal could be characterized as being three years of age or older, has been infected for two or more years and has persistent lymphocytosis. Special emphasis was placed on the inability to demonstrate BLV in normal semen and the inability of infectious leukocytes to survive manipulations such as freezing, a standard procedure in processing semen for artificial insemination. Studies on the response of lymphocytes to mitogens led to the conclusion that most BLV infected cattle have normal numbers of T lymphocytes and some may have an increase in the number of T lymphocytes. This suggests that BLV does not induce a deficiency in cell mediated immunity.

Dr. Lyle Miller, Iowa State University, Ames, IA, reported on studies to investigate the potential for virus transmission from cattle in which the results of repeated serologic tests were inconsistent. So far, all cattle that were seronegative at the time they were used as donors have proven to be free of infectious BLV.

The committee approved three resolutions relating to bluetongue. The resolutions addressed the need for surveillance and an economic impact statement for bluetongue serotype 2, the need for further work on bluetongue vaccine production and testing, and recognition of the contributions of scientists retiring from the Denver Bluetongue Laboratory.

The meeting was adjourned at 5:15 p.m.
ECONOMIC AND EPIDEMIOLOGIC ANALYSIS OF U.S. BOVINE BRUCELLOSIS PROGRAMS*
Raymond A. Dietrich, Ph.D.; Stephen H. Amosson, Ph.D.;
and Richard P. Crawford, DVM, Ph.D.**

The Cooperative State-Federal Brucellosis Eradication Program, which was established in 1934, has been highly successful in reducing brucellosis infection in U.S. cattle herds. Bovine brucellosis reactors as identified by the market cattle identification (MCI) program decreased from .97% of the total cattle tested in 1966 to .31% in 1984. Further, initial follow-up tests of BRT suspicious herds found infected declined from 1,653 in 1967 to 197 in 1984.

While the above data represents program progress in all dimensions, U.S. Department of Agriculture data indicated that bovine brucellosis infection was still present in 31 of the 50 states as of April 16, 1985. Given the wide variation in reactor rates between the non-Class Free states and the concentration of infection in nine states where 90% or more of the infection in the U.S. exists, it is essential that current and alternative bovine brucellosis programs are analyzed periodically to assure that the most economical and epidemiologically efficient programs are being utilized. This was recognized by the National Brucellosis Technical Commission (NBTC) in 1978 as evidenced by their statement that "investment in funds in epidemiologically sound modifications of the present program specifically targeted to varying requirements of herds, states and regions will produce a favorable return." Important herd, resource and management characteristics, however, vary not only by region but also within states, and strongly influence the types of control programs best suited to particular areas. The purpose of this research was to analyze the economic and epidemiologic impact of specified alternative bovine brucellosis programs and to provide a benefit-cost analysis of these alternative brucellosis control and/or eradication strategies for Veterinary Services, Animal & Plant Health Inspection Service (APHIS), U.S. Department of Agriculture. These strategies and/or alternative brucellosis programs were examined in terms of their costs and benefits to society, consumers, producers, and related agricultural industries. In addition, decision criteria such as change in benefits, change in program costs, and net benefits were estimated to provide guidelines to decision-makers concerning optimum bovine brucellosis control and/or eradication programs.

* Details concerning the methodology, research assumptions, input data, coefficients, research results and analysis of this presentation are in "Economic and Epidemiologic Analysis of U.S. Bovine Brucellosis Programs", a contract report prepared for VS, APHIS, USDA, Hyattsville, MD, August, 1985, by the Texas Agricultural Experiment Station, College Station, TX 77843.

**Respectively, Texas Agricultural Experiment Station, Department of Agricultural Economics, formerly Department of Agricultural Economics, and Department of Veterinary Public Health, Texas A&M University, College Station, Texas 77843.
METHODOLOGY

BRUSIM, a disease simulation model was developed to measure the impact of various program components upon selected epidemiologic parameters and for determining costs and physical losses associated with brucellosis control/eradication programs given epidemiologic coefficients and economic criteria from 1976 through 2005. The U.S. was delineated into 16 regions based upon such factors as brucellosis prevalence, producer characteristics and cattle population. Specific parameters relating to herd characteristics, herd management, epidemiology, and physical losses used in BRUSIM for the beef and dairy sectors are shown in Table 1.

TECHISM, an econometric model, was used for determining the net benefits accruing to society, consumers, producers, and related industries as a result of changes in beef and milk losses from alternative programs compared to a base program. The discounted values and associated program costs were used for determining benefit/cost ratios and related economic decision criteria.

Data for this study were obtained from both primary and secondary sources. Some of the basic information and epidemiologic data were obtained from the 1978 NBTC study. The NBTC data were supplemented and updated by information from the U.S. Department of Agriculture and U.S. Department of Commerce. Additional epidemiological data were obtained from a national survey of quarantined herd owners and a survey of quarantined and non-quarantined Texas producers. Data sources also included the expert judgement of NBTC personnel, brucellosis epidemiologists, and state and federal program officials.

The epidemiological model developed for this research contains (1) a beef sector and (2) a dairy sector. Results were simulated on a national and regional basis for each beef and dairy sector as follows (1) an epidemiological summary, (2) a brucellosis program test summary, (3) a non-primary surveillance summary, (4) brucellosis livestock producer expenditures, and (5) state and federal expenditures. Data and information from APHIS, U.S. Department of Agriculture, were used to validate the epidemiological parameters and output such as quarantined herds, reactors within quarantined herds, on farm tests, MCI cattle tests, calfhood vaccination, and state-federal program costs.

PROGRAM SCENARIOS ANALYZED

A base program and eight alternative bovine brucellosis programs were simulated for the contiguous 48 states. Prime consideration in the selection of program alternatives included potential or most likely industry requirements and potential federal funding for brucellosis programs. The nine programs simulated from fiscal year (FY) 1976 to FY 2005 included the following:

(1) Base Program — Defined as the FY-76 through FY-84 brucellosis program with changes in program procedures that would result in the disease remaining at relatively constant level from 1985 to 2005 to serve
Table 1. Bruism Input Factors as Related to Region, Herdsize, Year of Infection and Quarantine, and Beef and Dairy Sector, 1984.

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<tr>
<th>INPUT MATRIX DESCRIPTION</th>
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<td>Proportion of replacements purchased</td>
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<tr>
<td>Total number of herds</td>
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<td>Number of undetected affected herds</td>
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<td>Quarantined herds</td>
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<td>Average number of cows per herd</td>
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<td>X</td>
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<tr>
<td>Undetected within herd infection rates</td>
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<td>X</td>
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<tr>
<td>Undetected infected clean-up rates</td>
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<td>Regional sales probability</td>
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<td>Producer test cost per cow</td>
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<td>Weighted population proportions</td>
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<td>Percent contact herds tested</td>
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<td>X</td>
</tr>
<tr>
<td>Herdsize management parameter</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Owner testing percentage</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Secondary Epidemiologic testing percentage</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Post-quarantine testing percentage</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

*Dimensioned by model year
as the basis from which changes in program efficiency could be measured for alternative bovine brucellosis programs.

(2) Current (1976–84) Program — Defined as the existing 1976–84 bovine brucellosis program in the contiguous 48 states. This program was simulated to reflect a continuation of progress which has been made between FY-76 and FY-84 through FY-2005.

(3) Rapid Eradication — This scenario was modeled under (a) theoretical and (b) realistic modes.

a. Theoretical mode — Assumed that obstacles relating to manpower and industry cooperation were nonexistent and that there would be strict adherence to the requirements of the Uniform Methods and Rules (UM&R).

b. Realistic mode — Assumed that manpower and industry cooperation obstacles will continue to exist but that there would be strict adherence to the requirements of the UM&R by program authorities.

(4) Base Program with a 25 Percent Increase in Program Efficiency in Class C Regions — This scenario assumed that 1984 base program assumptions would apply to all regions from 1985 to 2005. It further assumed that there would be a 25 percent increase in program efficiency in terms of adherence to the UM&R after 1984 in Class C regions.

(5) Base Program with a 25 Percent Decrease in Program Efficiency in Class C Regions — This scenario assumed that 1984 base program assumptions would apply to all regions from 1985 to 2005. It further assumed that there would be a 25 percent decrease in program efficiency in terms of adherence to the UM&R after 1984 in Class C regions.

(6) No State-Federal Program with no vaccination — This scenario assumed that there would be a milk ordinance enforced brucellosis program in dairy cattle.

(7) No State-Federal Program with Calfohood Vaccination Supported by Industry — Two scenarios were modeled. Both scenarios assumed that annual federal funding will be limited to $6 million. Both also assumed that there would be a milk ordinance enforced brucellosis program in cattle. One scenario assumed that vaccination would result in a 45% vaccination level of female calves entering the herd and the other assumed a 75% vaccination level of female calves entering the herd.

RESULTS

Major findings resulting from the alternative bovine brucellosis programs analyzed in this study are as follows with respect to:

(1) Control and/or Eradication of Brucellosis Infection. Four programs, the theoretical eradication program, the realistic eradication program, the base program with a 25% increase in efficiency in Class C regions, and the current program were highly effective in reducing bru-
Table 2. Percentage Change in Quarantined Herds, Quarantined Infected Cows, Undetected Infected Herds, Undetected Infected Cows, Total Infected Cows, Weaner Calf and Milk Losses, and Total Costs, By Alternative Bovine Brucellosis Program, United States, FY 1984 to FY 2005.\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Current Program</th>
<th>Realistic Eradication</th>
<th>Theoretical Eradication</th>
<th>Baseline - 25% Increase In Efficiency In C Regions</th>
<th>Baseline - 25% Decrease In Efficiency In C Regions</th>
<th>No Program</th>
<th>No Program With 45% Calfhood Vaccination</th>
<th>No Program With 75% Calfhood Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarantined Herds</td>
<td>-89.2</td>
<td>-99.2</td>
<td>-99.9</td>
<td>-94.9</td>
<td>-18.4</td>
<td>27,079.8(^2)</td>
<td>4,909.4(^2)</td>
<td>701.7(^2)</td>
</tr>
<tr>
<td>Quarantined Cows</td>
<td>-83.5</td>
<td>-99.8</td>
<td>-100.0(^3)</td>
<td>-92.5</td>
<td>22.3</td>
<td>32,314.8(^2)</td>
<td>4,747.7(^2)</td>
<td>504.3(^2)</td>
</tr>
<tr>
<td>Undetected Infected Herds</td>
<td>-86.3</td>
<td>-99.4</td>
<td>-100.0</td>
<td>-93.9</td>
<td>80.0</td>
<td>4,887.9</td>
<td>2,264.4</td>
<td>775.7</td>
</tr>
<tr>
<td>Undetected Infected Cows</td>
<td>-82.0</td>
<td>-99.2</td>
<td>-100.0(^3)</td>
<td>-92.7</td>
<td>163.7</td>
<td>15,505.4</td>
<td>5,021.0</td>
<td>1,209.3</td>
</tr>
<tr>
<td>Total Infected Cows</td>
<td>-82.9</td>
<td>-99.5</td>
<td>-100.0(^3)</td>
<td>-92.5</td>
<td>78.0</td>
<td>6,926.2</td>
<td>2,049.2</td>
<td>432.3</td>
</tr>
<tr>
<td>Weaner Calf Losses</td>
<td>-83.1</td>
<td>-99.5</td>
<td>-100.0(^3)</td>
<td>-92.5</td>
<td>83.2</td>
<td>6,101.4</td>
<td>1,891.6</td>
<td>390.6</td>
</tr>
<tr>
<td>Milk Losses</td>
<td>-80.5</td>
<td>-97.8</td>
<td>-99.7</td>
<td>-90.5</td>
<td>1.6</td>
<td>36,194.1</td>
<td>5,272.4</td>
<td>558.8</td>
</tr>
<tr>
<td>Total Costs</td>
<td>-19.2</td>
<td>-50.1</td>
<td>-39.5</td>
<td>-22.6</td>
<td>1.9</td>
<td>-76.4</td>
<td>-65.8</td>
<td>-47.6</td>
</tr>
</tbody>
</table>

1) 1984 baseline simulation results for the above parameters were as follows. quarantined herds, 8,467; quarantined cows, 88,756; undetected infected herds, 12,008; undetected infected cows, 57,724; total infected cows, 146,480, weaner calf losses, 19,454,818 (pounds); dairy milk losses, 98,680 (hundredweight); and total costs, $168,894,576.

NOTE: Quarantined herds and cows for the No Program scenarios reflects identified infected dairy herds and dairy cows only.

2) Reflects changes in identified infected dairy herds and cows only after 1984. Quarantined dairy herds and cows in 1984 were 297 and 3,961, respectively.

3) Less than .005 percent.
The theoretical eradication program demonstrated that eradication could be achieved within 3 to 5 years when program constraints are eliminated with the only limiting factors being the current state of technology. These results demonstrate that the current state of technology is sufficient to achieve eradication, with the time frame being dependent upon financial and manpower commitment, provided program efficiency is maintained at high levels, producer and agency cooperation is maximized, and depopulation of infected herds is judiciously applied. These research results parallel the recent field experience of the Canadian Department of Agriculture which reported no known bovine brucellosis infection in Canada since March 1984 after initiating a strong market cattle testing program, along with an indemnity and depopulation program which encouraged producer cooperation in 1979.

The realistic eradication program, which assumed 1982-84 funding levels, strict adherence to the UM&R by program authorities, and a modified depopulation scheme, reduced total infected cows by more than 92% from 1984 to 1990. Increased emphasis on depopulation of detected herds, although increasing program costs in the realistic eradication program, would likely have decreased infected cows at a faster rate than reported above. The net results are that the realistic eradication program would be a powerful tool leading to eradication given increased producer cooperation via incentives or educational programs plus producer incentives for depopulating known infected herds.

The base program with a 25% increase in program efficiency in Class C regions, although revealing infection levels ranging from 5 to 7% higher in all infection parameters than did the realistic eradication program by 2005, was more effective in reducing infection than the current program. Results reveal that a 25% increase in program efficiency in high incidence or Class C regions through stricter adherence to the UM&R or other incentives would be highly effective in reducing infection levels.

The base program with a 25% decrease in program efficiency in Class C regions demonstrated that reductions in program efficiency in high incidence regions would result in substantial increases in infection. The most dramatic increases in bovine brucellosis infection were generated by the three no state-federal program scenarios. Total infected cows increased 69 fold under the no state-federal program without calfhood vaccination from FY 1984 to 2005 compared to a 4 fold increase in infected cows for the no program scenario with a 75% calfhood vaccination level. These results demonstrate that (1) calfhood vaccination would be highly beneficial under a no state-federal program scenario or when bovine brucellosis infection exists at relatively high levels, and (2) no state-federal programs with calfhood vaccination, even at high levels of calfhood vaccination, were substantially inferior with respect to reducing bovine brucellosis infection compared to other alternative programs simulated.

(2) Reduction of Physical Losses. Weaner calf and milk losses were
reduced most effectively by the theoretical eradication program, followed closely by the realistic eradication program, Tables 3 and 4. The base program with a 25% increase in program efficiency ranked third in reducing physical losses followed by the current program. All other program efficiency in Class C regions and the three no program scenarios increased physical losses with the sharpest increases occurring under the no program scenario.

(3) **Program Costs.** Total discounted program costs over the 30 year period simulated were lowest for the no state-federal program without calfhood vaccination followed by the no state-federal program with calfhood vaccination, Table 5. Costs accruing to these programs were attributable to producer costs associated with a milk ordinance enforced brucellosis program in dairy cattle and calfhood vaccination. The highest program cost occurred under the base program with a 25% decrease in program efficiency in Class C regions due to additional secondary epidemiologic tracing, adjacent herd testing, and herd testing as infection increased.

The lowest total program costs associated with alternative programs most effective in reducing brucellosis infection and physical losses were almost identical at $1.7 billion for the realistic and theoretical eradication programs as were net declines or savings in program costs at more than $400 million for both eradication programs compared to the base program. The base program with a 25% increase in program efficiency in Class C regions and the current program ranked third and fourth, respectively, in total costs and declines in costs for those programs most effective in reducing infection and physical losses. Net declines in total costs accrue from lower program costs attributable to program efficiency resulting in fewer number of secondary epidemiologic traces, adjacent herd tests, quarantine and post-quarantine herd tests, and handling costs.

(4) **Economic Benefits.** The highest positive change in benefits to society, net change in benefits to society, and benefit/cost ratios accrued from the theoretical eradication program, followed closely by the realistic eradication program, Tables 5 and 6. The base program with a 25% increase in program efficiency and the current program ranked third and fourth respectively, relative to positive changes in benefits and benefit/cost ratios. The three no state-federal program scenarios and the base program with a 25% decrease in program efficiency in Class C regions all produced negative changes in benefits to society, compared to the base program, as well as generating economically unacceptable benefit/cost ratios.

(5) **Equity Impact.** Equity analysis revealed that consumers would accrue substantial positive benefits from programs which decrease infection as the eradication programs followed by the base program with a 25% increase in efficiency in Class C regions and the current program, Table 7. Further, consumers would incur large negative benefits or losses from programs which increase infection as the no state-federal program sce-
Table 3. Beef and Dairy Weaner Calf Losses, By Program, United States, 1976–2005

<table>
<thead>
<tr>
<th>Year</th>
<th>Baseline Program</th>
<th>Current Program</th>
<th>Baseline – 25% Increase in C Regions</th>
<th>Baseline – 25% Decrease in C Regions</th>
<th>No Program</th>
<th>No Program With 45% Calfhood Vaccination</th>
<th>No Program With 75% Calfhood Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>47,029</td>
<td>47,029</td>
<td>47,029</td>
<td>47,029</td>
<td>47,029</td>
<td>47,029</td>
<td>47,029</td>
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<tr>
<td></td>
<td>579</td>
<td>579</td>
<td>579</td>
<td>579</td>
<td>579</td>
<td>579</td>
<td>579</td>
</tr>
<tr>
<td></td>
<td>47,608</td>
<td>47,608</td>
<td>47,608</td>
<td>47,608</td>
<td>47,608</td>
<td>47,608</td>
<td>47,608</td>
</tr>
<tr>
<td>1980</td>
<td>31,782</td>
<td>31,782</td>
<td>31,782</td>
<td>31,782</td>
<td>31,782</td>
<td>31,782</td>
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<td>252</td>
<td>252</td>
<td>252</td>
<td>252</td>
<td>252</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>32,034</td>
<td>32,034</td>
<td>32,034</td>
<td>32,034</td>
<td>32,034</td>
<td>32,034</td>
<td>32,034</td>
</tr>
<tr>
<td>1985</td>
<td>15,993</td>
<td>16,295</td>
<td>13,296</td>
<td>6,892</td>
<td>16,659</td>
<td>16,047</td>
<td>22,790</td>
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<tr>
<td></td>
<td>80</td>
<td>72</td>
<td>68</td>
<td>62</td>
<td>73</td>
<td>78</td>
<td>312</td>
</tr>
<tr>
<td></td>
<td>16,073</td>
<td>16,367</td>
<td>13,364</td>
<td>6,954</td>
<td>16,732</td>
<td>16,125</td>
<td>22,790</td>
</tr>
<tr>
<td>1990</td>
<td>13,233</td>
<td>7,030</td>
<td>1,412</td>
<td>27</td>
<td>3,324</td>
<td>14,833</td>
<td>48,386</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>40</td>
<td>16</td>
<td>2</td>
<td>25</td>
<td>69</td>
<td>48,386</td>
</tr>
<tr>
<td></td>
<td>13,391</td>
<td>7,070</td>
<td>1,428</td>
<td>29</td>
<td>3,349</td>
<td>14,902</td>
<td>48,386</td>
</tr>
<tr>
<td>1995</td>
<td>15,971</td>
<td>5,540</td>
<td>548</td>
<td>4</td>
<td>2,170</td>
<td>19,978</td>
<td>111,838</td>
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<td></td>
<td>69</td>
<td>29</td>
<td>6</td>
<td>4</td>
<td>16</td>
<td>76</td>
<td>1,149</td>
</tr>
<tr>
<td></td>
<td>16,040</td>
<td>5,569</td>
<td>554</td>
<td>4</td>
<td>2,185</td>
<td>20,054</td>
<td>112,987</td>
</tr>
<tr>
<td>2000</td>
<td>16,019</td>
<td>3,752</td>
<td>142</td>
<td>1</td>
<td>1,420</td>
<td>23,429</td>
<td>211,223</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>22</td>
<td>3</td>
<td>2</td>
<td>11</td>
<td>86</td>
<td>2,549</td>
</tr>
<tr>
<td></td>
<td>16,090</td>
<td>3,774</td>
<td>145</td>
<td>1</td>
<td>1,431</td>
<td>23,515</td>
<td>213,772</td>
</tr>
<tr>
<td>2005</td>
<td>20,519</td>
<td>3,275</td>
<td>88</td>
<td>2</td>
<td>1,427</td>
<td>35,535</td>
<td>382,437</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>101</td>
<td>94,852</td>
</tr>
<tr>
<td></td>
<td>20,592</td>
<td>3,291</td>
<td>90</td>
<td>1</td>
<td>1,436</td>
<td>35,636</td>
<td>387,470</td>
</tr>
</tbody>
</table>

1) Data by year and program reflect beef, dairy and total, respectively. Losses are on a thousand pound basis.
2) Less than 500 pounds.
Table 4. Dairy Milk Losses, By Program, United States, 1976–2005\textsuperscript{11}

<table>
<thead>
<tr>
<th>Year</th>
<th>Baseline Program</th>
<th>Current Program</th>
<th>Realistic Eradication</th>
<th>Theoretical Eradication</th>
<th>Baseline – 25% Increase in Efficiency in C Regions</th>
<th>Baseline – 25% Decrease in Efficiency in C Regions</th>
<th>No Program</th>
<th>No Program With 45% Calfhood Vaccination</th>
<th>No Program With 75% Calfhood Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>87,267</td>
<td>80,737</td>
<td>77,336</td>
<td>75,884</td>
<td>82,200</td>
<td>85,447</td>
<td>293,039</td>
<td>293,039</td>
<td>293,039</td>
</tr>
<tr>
<td>1990</td>
<td>73,527</td>
<td>45,744</td>
<td>17,950</td>
<td>1,872</td>
<td>27,258</td>
<td>73,544</td>
<td>1,230,802</td>
<td>505,187</td>
<td>220,145</td>
</tr>
<tr>
<td>1995</td>
<td>74,488</td>
<td>33,802</td>
<td>7,255</td>
<td>422</td>
<td>15,913</td>
<td>78,832</td>
<td>5,320,266</td>
<td>1,202,901</td>
<td>289,916</td>
</tr>
<tr>
<td>2000</td>
<td>75,844</td>
<td>27,004</td>
<td>2,866</td>
<td>310</td>
<td>11,222</td>
<td>87,157</td>
<td>16,080,474</td>
<td>2,639,852</td>
<td>424,144</td>
</tr>
<tr>
<td>2005</td>
<td>76,099</td>
<td>19,235</td>
<td>2,124</td>
<td>299</td>
<td>9,360</td>
<td>100,226</td>
<td>35,815,037</td>
<td>5,301,481</td>
<td>650,074</td>
</tr>
</tbody>
</table>

\textsuperscript{11} Milk losses are in hundredweight.
Table 5. Summary of Program Alternatives, By Selected Criteria, Compared to the Base Program.¹¹

<table>
<thead>
<tr>
<th>Program Alternative</th>
<th>Change in Producer and Consumer Benefits</th>
<th>Total Program Costs</th>
<th>Total Annual Increase in Program Costs</th>
<th>Total Annual Decrease in Program Costs</th>
<th>Net Change in Program Costs</th>
<th>Net Change in Producer and Consumer Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>473.50</td>
<td>2,089.33</td>
<td>27.33</td>
<td>94.98</td>
<td>-67.65</td>
<td>540.96</td>
</tr>
<tr>
<td>Realistic Eradication</td>
<td>733.12</td>
<td>1,732.60</td>
<td>45.16</td>
<td>469.54</td>
<td>-424.38</td>
<td>1,157.50</td>
</tr>
<tr>
<td>Theoretical Eradication</td>
<td>837.70</td>
<td>1,728.69</td>
<td>41.60</td>
<td>469.89</td>
<td>-428.29</td>
<td>1,265.99</td>
</tr>
<tr>
<td>Baseline – 25% Increase in Efficiency In Class C Regions</td>
<td>636.05</td>
<td>2,032.54</td>
<td>30.52</td>
<td>154.96</td>
<td>-124.44</td>
<td>760.49</td>
</tr>
<tr>
<td>Baseline – 25% Decrease in Efficiency In Class C Regions</td>
<td>-206.15</td>
<td>2,203.55</td>
<td>47.88</td>
<td>1.31</td>
<td>46.57</td>
<td>-252.72</td>
</tr>
<tr>
<td>No Program</td>
<td>-18,338.17</td>
<td>447.14</td>
<td>0</td>
<td>1,709.84</td>
<td>-1,709.84</td>
<td>-16,628.33</td>
</tr>
<tr>
<td>No Program With 45% Calfhood Vaccination</td>
<td>-5,429.78</td>
<td>872.69</td>
<td>0</td>
<td>1,284.29</td>
<td>-1,284.29</td>
<td>-4,145.49</td>
</tr>
<tr>
<td>No Program With 75% Calfhood Vaccination</td>
<td>-1,387.93</td>
<td>1,267.32</td>
<td>0</td>
<td>889.66</td>
<td>-889.66</td>
<td>-498.27</td>
</tr>
</tbody>
</table>

¹¹ Dollars are in 1982 real dollars along with a 4 percent real discount rate. Total program costs for the base program were $2,156.98 million.
Table 6. Ranking of Program Alternatives, By Selected Criteria, Compared to the Base Program

<table>
<thead>
<tr>
<th>Program Alternative</th>
<th>Change In Producer and Consumer Benefits</th>
<th>Net Change In Producer and Consumer Benefits</th>
<th>Change In Program Costs</th>
<th>Regular Benefit/Cost Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Program</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Realistic Eradication</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Theoretical Eradication</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Baseline – 25% Increase in Efficiency In Class C Regions</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Baseline – 25% Decrease in Efficiency In Class C Regions</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>No Program</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>No Program With 45% Calfhood Vaccination</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>No Program With 75% Calfhood Vaccination</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

* Benefit/cost ratios were as follows: current program 1.26, realistic eradication 1.68, theoretical eradication 1.73, baseline – 25% increase in efficiency in Class C regions 1.11, baseline – 25% decrease in efficiency in Class C regions 0.89, no program – 36.19, no program with 45% CV – 3.75, and no program with 75% CV 6.1.
Table 7. Change in Benefits From Weaner Calf Losses Which Accrue To Consumers, Livestock Producers, Related Agricultural Industries And Total Benefits, By Program Alternative Compared To The Base Program, United States

<table>
<thead>
<tr>
<th>Program Alternative</th>
<th>Change In Consumer Benefits</th>
<th>Change In Livestock Producer Benefits</th>
<th>Change In Related Agricultural Industry Benefits</th>
<th>Change In Total Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current</td>
<td>552.64</td>
<td>-225.18</td>
<td>140.13</td>
<td>467.59</td>
</tr>
<tr>
<td>Realistic Eradication</td>
<td>848.63</td>
<td>-345.50</td>
<td>220.23</td>
<td>723.36</td>
</tr>
<tr>
<td>Theoretical Eradication</td>
<td>961.01</td>
<td>-391.06</td>
<td>256.11</td>
<td>826.06</td>
</tr>
<tr>
<td>Baseline - 25% Increase In Efficiency In Class C Regions</td>
<td>724.31</td>
<td>-295.00</td>
<td>198.47</td>
<td>627.78</td>
</tr>
<tr>
<td>Baseline - 25% Decrease In Efficiency In Class C Regions</td>
<td>-280.07</td>
<td>114.40</td>
<td>-39.63</td>
<td>-205.30</td>
</tr>
<tr>
<td>No Program</td>
<td>-23,905.03</td>
<td>9,564.62</td>
<td>-2,620.34</td>
<td>-16,960.75</td>
</tr>
<tr>
<td>No Program With 45% Calfhood Vaccination</td>
<td>-7,271.71</td>
<td>2,952.50</td>
<td>-866.82</td>
<td>-5,186.03</td>
</tr>
<tr>
<td>No Program With 75% Calfhood Vaccination</td>
<td>-1,810.54</td>
<td>737.68</td>
<td>-269.93</td>
<td>-1,342.79</td>
</tr>
</tbody>
</table>
narios. Benefits to related agricultural industries paralleled those of consumers but at a lower level. Although producers with infected herds may incur catastrophic losses, programs which decrease infection increase supplies of meat and milk, exert a downward pressure on price, creating negative benefits to producers. However, summation of benefits over all sectors revealed that substantial benefits would accrue to society from alternative programs which decrease infection, as the eradication programs, and that large negative benefits would accrue to society from alternative programs which increased infection and physical losses.

EPIDEMIOLOGIC AND ECONOMIC CONSIDERATIONS

Some epidemiologic and economic considerations emanating from this study may be summarized as follows:

(1) The realistic eradication program appears to be the most effective program of the alternative programs analyzed since it ranked above other alternative programs, except the theoretical eradication program, in total benefits, net benefits, and benefit cost ratios. The theoretical eradication program demonstrated that the present "state of the arts" within the U.S. bovine brucellosis program is capable of detecting sufficient numbers of infected herds for achieving eradication. Application and/or utilization of current program components at higher efficiency levels through stricter adherence to the requirements of the UM&R and continuation of producer incentives via indemnity payments, along with the incorporation of a depopulation program, would likely result in an annual increase in program costs for a short interim period over the program costs currently incurred. However, simulation results of this study, as well as the recent experience of the Canadian Department of Agriculture, suggest that such an approach would be cost effective while leading toward the goal of eradication.

(2) Joint consideration of economic and epidemiologic efficiency is paramount to animal disease programs as bovine brucellosis, given the wide variation in infection among and between states as evidenced by the bovine brucellosis classification system, and as states reach or approach class free status. Future cost considerations may suggest, for example, that program officials and agencies concerned with determining program policies give continuing periodic consideration to adjusting program components on a regional or national basis, given prevailing infection levels, while adhering to the requirements of an epidemiologically sound and efficient disease monitoring system and overall program objective.

(3) Prior research by Amosson revealed that utilization of contact or adjacent herd testing was an important epidemiologic tool and highly cost effective in detecting and eliminating brucellosis infection. Results of this study suggest that maximization of adjacent herd testing, post quarantine and post ownership testing, and secondary epidemiologic tracing efficiencies, along with an efficient primary surveillance system as MCI and BRT, is essential in all area classification systems if bovine brucellosis is to be eradicated.
(4) Simulation results again revealed the importance of calfhood vaccination in combating brucellosis infection in high incidence regions as under the no state-federal program scenario where benefits of calfhood vaccination greatly exceeded costs. However, utilization of calfhood vaccination in low incidence regions is not cost effective plus it tends to inhibit the efficiency of the MCI program. This study further demonstrated that calfhood vaccination, by itself or in the absence of other current program components, will keep brucellosis infection from spreading as rapidly as it would in the absence of vaccination, but calfhood vaccination alone did not keep brucellosis infection from increasing.

(5) Results of the study suggest that additional research may be highly beneficial for establishing economic and epidemiologic guidelines relative to depopulation policies, application of adjacent herd testing to immediate contact herds or on a broader basis, post quarantine testing, and changes in other primary and secondary program components if eradication is to be achieved in the most economical and epidemiologically efficient manner.

(6) Equity analysis revealed that consumers were the major beneficiaries of investments in publicly funded bovine brucellosis programs, which decreased physical losses and increased supplies of meat and milk.

REFERENCES

USE OF ELISA IN PREDICTING LATENT CALFHOOD BRUCELLOSIS INFECTION
Scott L. Reynolds, DVM, MSPH, James L. Lindstrom, DVM and Thurman L. Fancher, DVM
Texas Animal Health Commission
Austin, Texas

INTRODUCTION

Earlier reports addressed the efficiency of Enzyme-Linked Immunosorbent Assay (ELISA) and Automated Complement Fixation Tests (ACF) to predict Brucella abortus infection in cows prior to and after inoculation with Strain 19 B. abortus vaccine. Animals showing suspect titers only on the ELISA were isolated and those animals subsequently exhibiting reactor level titers by ACF tests were removed to slaughter. The ELISA demonstrated a greater efficiency than the ACF in predicting brucellosis.¹

Latent calfhood infection has been well documented in heifers born to cows in infected herds. In an earlier report, two latent calfhood infected heifers were detected by the ELISA and isolated seven months prior to exhibiting reactor level titers on the ACF test. One animal was the offspring of a reactor mother. The other heifer was born to a mother showing negative serological titers. Both heifers were slaughtered and revealed culture positive isolates of B. abortus.²

This report addresses the efficiency of ELISA and ACF tests to predict B. abortus in heifers born to cows in herds infected with B. abortus.

ANIMALS

Three commercial heifer herds were used for this study. Herd "A" was "put together" crossbred Brangus herd containing animals originating from five southern states. The herd was calfhood vaccinated at an unknown age and at 11 months of age was sold to the present owner through a broker. At an average of 13 months, the heifers were put with a bull. Seven months later, in preparation for sale, all animals were tested for B. abortus and 14 reactors were revealed. Three animals were slaughtered and lymph nodes cultured. B. abortus was isolated from all three animals. Herds "B" and "C" were "home raised" crossbred Brahma heifers. Both herds were offspring of the same herd of cows but born 12 months apart. The parent herd, as described in an earlier report, contained a total of 693 mother cows on four pastures.¹ A total of 245 reactors were removed from this herd at the time of adult vaccination. Ten of these reactors, as well as 17 additional animals, aborted their calves during a four month period prior to adult vaccination. The parent herd experienced 23 additional reactors prior to quarantine release. Heifers in Herds "B" and "C" were born during the months of January through May and were weaned the following November. At weaning, all heifers were inoculated with Strain 19 B. abortus vaccine and isolated as a separate herd. Herd "B" as 12 to 17 months of age at the time the mother herd was adult vaccinated and reactors were removed.

STUDY DESIGN

...
USE OF ELISA IN PREDICTING LATENT CALFHOOD

vaccine and isolated as a separate herd. Herd “B” was 12 to 17 months of age at the time the mother herd was adult vaccinated and reactors were removed.

Herd “C” at the same time period consisted of heifers less than six months of age. Therefore, Herd “B” was born in the spring and winter of 1982 and Herd “C” during the same time period but in 1983.

ANIMAL MANAGEMENT

Serum from heifers was studied by the ACF, ELISA and Card tests at every test period. The Rivanol test was performed only on card positive animals. Heifers showing suspect titers only on the ELISA were isolated and those animals subsequently exhibiting reactor level titers by ACF tests were removed to slaughter.

Herd “A” was initially tested at approximately 20 months of age. At this time, all animals were palpated and divided into open, main and ELISA suspect herds. The main herd was retested in 48 days and at 15 to 100 day intervals thereafter. The ELISA suspect herd was tested at 15 day intervals. The open herd was shipped to slaughter.

Herd “B” was initially tested between 18 and 23 months of age. The herd was retested in 210 days and at 60 to 210 day intervals thereafter. Herd “B” was palpated at the time of the initial test. The herd was separated into an open and bred herd.

Herd “C” was initially tested at five to nine months of age. At this time the heifers were vaccinated against brucellosis and weaned. The herd was retested in 210 days and at 60 to 270 day intervals thereafter.

At this time Herd “A” has been studied over a ten month period. Herds “B” and “C” over periods of 20 and 24 months, respectively. When available, lymph nodes have been harvested on slaughtered animals and bacteriologically assayed for B. abortus.

CULTURE TECHNIQUE

The suprapharyngeal, mandibular, supramammary and internal iliac lymph nodes were removed. All tissues were packed in whirlpacks and stored immediately in ice until received at the laboratory. Lymph nodes were removed from surrounding fat, dipped in alcohol, flamed, and sliced. The cut surface was minced and rubbed over the surface of the medium. All tissues were assayed according to the procedures utilized by the National Veterinary Services Laboratory in Ames, Iowa, except Farrel’s media was substituted for W media.

CONVENTIONAL SEROLOGICAL TESTS

The Card and Rivanol tests were performed by the State/Federal Diagnostic Laboratory, Austin, Texas and interpreted as prescribed in the Brucellosis Eradication Uniform Methods and Rules (UM&R).

AUTOMATED COMPLEMENT FIXATION TEST

The Technicon Auto Analyzer II, located at the State /Federal Diagnos-
tic Laboratory, Austin, Texas was used for ACF. A detailed description of reagents may be found in the Technicon Instruction Manual on “Automated Complement Fixation Testing.” Diagrams of equipment and flow of serum and reagents have been pictured in detail. ACF results were interpreted according to the following standard: $20^+\text{ or greater} = \text{reactor}$, $10^+ = \text{suspect}$, and $5^+ \text{ or less} = \text{negative}$.

**ENZYME-LINKED IMMUNOSORBENT ASSAY TEST**

The ELISA was performed at the State/Federal Diagnostic Laboratory, Austin, Texas. Equipment utilized for these studies was manufactured by Dynatech, Alexandria, Virginia. This equipment consisted of the following: Dynatech MR 600 microplate reader, Dynatech Microshaker, Dynawasher II, dynadrops dispenser and Immulon II microtiter plates. Standards for the ELISA are as follows: Spectrophotometric absorbance values ($\text{SAV } 0.000 \text{ to } 0.599 = \text{negative}, \text{SAV } 0.60 \text{ to } 0.99 = \text{suspect} \text{ and } \text{SAV } 1.00 \text{ or greater} = \text{positive}$). For this study, all suspect and positive animals were classified as suspect. A detailed description of reagents and procedures are described by Heck.\textsuperscript{3,4}

**RESULTS**

All culture positive isolates were identified as Bio Type I, \textit{B. abortus}. Positive isolates were made from lymph nodes of 32 heifers in Herd “A”. Two other heifers in Herd “A” yielded cultures negative for \textit{B. abortus}. Three positive isolates were made from three heifers in Herd “B”.

Table I summarizes the results of testing Herd “A” through 300 days. Note that after testing on day zero the main herd contained only bred heifers. As can be seen, a comparison is made of the number of reactors revealed between the main herd and the ELISA suspect herd. Those animals in the ELISA suspect herd remained isolated until after parturition. Animals showing reactor level titers on conventional tests were removed to slaughter. Those heifers negative on conventional tests were removed to the cow-calf herd. A total of 11 reactors not previously detected by the ELISA were removed from the main herd. Conversely, 20 reactors were removed from the ELISA suspect herd. Of these 20 animals, 19 showed positive bacteriological cultures for Bio Type I, \textit{B. abortus}. These animals were isolated prior to calving. No reactors were removed from the main herd after the 123 day test period. It is further noted that the ELISA suspect herd showed no reactors after the 183 day test period. After this test period, all bred cows had calved. Note that in column four, there are 16 ELISA suspects remaining in the herd after 300 days of testing. As previously stated, all animals at this time period had calved. It is postulated that ELISA antibody activity is the result of residual antibody and not indicative of brucellosis. Not shown on Table 1 are six reactors and seven ELISA suspects revealed in the open herd (62 animals). The open herd was retested 60 days after the initial test and sent to slaughter.

Table 2 summarizes the use of the ELISA and ACF tests to predict \textit{B. abortus} in heifers heavy bred at the time of removal to slaughter or
isolation. The column on the right shows that there were five heifers exhibiting suspect titers on the Elisa, but undetected by the ACF tests. The middle column shows those heifers exhibiting reactor level titers at the time of removal. These animals were negative on all serological tests at the previous test period. A total of five bred animals were predicted by the ELISA to be reactors at the time of isolation. These animals subsequently became reactors while in isolation. Conversely, nine bred animals previously undetected by the ELISA were sent to slaughter as reactors. While en route to slaughter, animal No. 175 delivered a weak calf that later died.

Table 3 compares the efficiency of the ACF and ELISA tests to predict B. abortus in heifers prior to parturition. The column on the right reflects the calving status of the heifers at the time of isolation or removal. As can be seen, 15 animals were suspect on the ELISA and isolated from the herd prior to calving. These animals subsequently became reactors and were removed to slaughter. Conversely, two reactors were removed after calving. These animals were not predicted by the ELISA on the previous test and, therefore, not isolated. It is of interest that animal No. 199 was detected as a reactor following a 48 day test period. For those animals removed by the ELISA prior to calving, the prediction time ranged from three to 66 days with an average of 46 days. Two of these early detected animals aborted while in isolation. One animal delivered a dead calf.

Table 4 summarizes Herd “B” through a 20 month test period. The herd consisted of 104 heifers at the time of initial testing. As can be seen, one reactor and four ELISA suspects were removed on the first test. It is of interest that these five animals were slaughtered by the owner and were not studied past this test period. None of these animals were available for culture assays. It is important to note that further testing revealed three additional reactors. These animals were previously detected by the ELISA test. No reactors were revealed by the main herd. Furthermore, the last reactor revealed experienced abortion while in isolation. Culture assays on all three ELISA suspects removed to slaughter showed positive isolates for B. abortus. One of these reactor heifers calved prior to removal from the main herd.

Table 5 summarizes the results of testing Herd “C” through a 24 month test period. A total of 22 ELISA suspects were detected and isolated. All animals were negative on all tests after 24 months of testing. It is of interest that those animals identified by the ELISA represent 22 different animals.

**DISCUSSION**

Testing of Herd “A” resulted in the removal and isolation of 17 reactors prior to the time calving commenced in the herd. These 17 heifers were identified by their vaccination tags as originating from four states and were postulated to represent latent calfhood infection. It is surmised the remaining 30 reactors resulted from latent as well as secondary infection. This assumption is based on the fact that two reactor animals calved in the herd prior to removal. Furthermore, the open herd, which experienced six
reactors, was removed from the bred herd prior to commencement of calving. Consequently, these open animals were also believed to be latent infected. Though the ELISA failed to detect two reactors prior to calving, those 20 early detected animals represent an important reduction of exposure. This assumption is further supported by the fact that two animals aborted while in isolation thereby further reducing exposure. No animals aborted while remaining in the main herd. Except for two animals, the ELISA identified potential reactors an average of 46 days prior to calving.

Herds “B” and “C” present an interesting paradox. Although offspring of the same herd, they were born 12 months apart, and presented vastly different disease pictures. Herd “B” experienced four reactors as well as seven ELISA suspects. Three of these reactors produced positive isolates for *B. abortus*. The remaining five animals were slaughtered but were unavailable for culture assays. Conversely, Herd “C” experienced 22 ELISA suspects, all of which were negative on all tests 150 days after the cessation of calving in the herd.

The number of infected cows in the parent herd was unknown at the time the animals in Herd “B” were born. It is believed the parent herd was infected prior to 1982. This is based on the fact that all heifers were weaned and isolated in November, thereby removing them from further exposure. No known infected herds were adjacent to either heifer herd. The mother herd was not diagnosed as having brucellosis until the following January when abortion was noted. Herd “B” represents those heifers remaining after culling in November 1982. Brucellosis was not diagnosed in the parent herd until 1983; consequently, culling was based on conformation and was unrelated to the infectious status of the mother. In contrast, Herd “C” contained only those animals born to noninfected mothers. As previously noted, all reactors and their female offspring were removed from the herd. The following factors are believed to have contributed to the lack of latent infection in Herd “C”:

1. Adult vaccination of parent herd thereby reducing exposure:
2. Removal of 91.4% of the infected cows when heifers were less than six months of age, further reducing exposure;
3. Removal of all heifers with their infected mothers; and,
4. Age resistance in heifers born to noninfected mothers.

The data further suggests that the use of the ELISA to test heifers from infected herds prior to calving is an important serodiagnostic tool in the management of brucellosis.

ACKNOWLEDGEMENTS

The author is grateful to Mary Menn, Roger Brasfield, Kathryn Miller and Rick Nabors, State/Federal Laboratory, Austin, Texas for their laboratory support. The author is also grateful to James Belcher, Texas Animal Health Commission, for his outstanding support in collection of tissue and to Ms. Toni Wayland for preparation of the manuscript.
REFERENCES


TABLE 1

SUMMARY OF RESULTS OF TESTING HERD "A" THROUGH 300 DAYS

<table>
<thead>
<tr>
<th>DAY #</th>
<th>MAIN HERD</th>
<th>REACTORS FOUND IN MAIN HERD</th>
<th>ELISA SUSPECT HERD</th>
<th>REACTORS FOUND IN SUSPECT HERD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>262*</td>
<td>14</td>
<td>16</td>
<td>N/T</td>
</tr>
<tr>
<td>15</td>
<td>N/T</td>
<td>N/T</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>48</td>
<td>196</td>
<td>4</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>53</td>
<td>N/T</td>
<td>N/T</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>78</td>
<td>187</td>
<td>3</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>108</td>
<td>183</td>
<td>2</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>123</td>
<td>178</td>
<td>3</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>138</td>
<td>99</td>
<td>0</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>153</td>
<td>52</td>
<td>0</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>168</td>
<td>39</td>
<td>0</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>183</td>
<td>35</td>
<td>0</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>197</td>
<td>0</td>
<td>N/T</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>N/T</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

*open cows were removed from main herd after this test period

N/T - No Test
TABLE 2

HERD "A"

COMPARISON OF ELISA AND ACF SERODIAGNOSTIC TESTS TO PREDICT BRUCELLA ABORTUS IN HEIFERS HEAVILY BRED AT THE TIME OF REMOVAL TO SLAUGHTER OR ISOLATION

<table>
<thead>
<tr>
<th>HEIFER ID #</th>
<th>TEST RESULTS AT TIME OF REMOVAL OR ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACF</td>
</tr>
<tr>
<td>118</td>
<td>-</td>
</tr>
<tr>
<td>173</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>104</td>
<td>-</td>
</tr>
<tr>
<td>52</td>
<td>-</td>
</tr>
<tr>
<td>44</td>
<td>+</td>
</tr>
<tr>
<td>119</td>
<td>+</td>
</tr>
<tr>
<td>211</td>
<td>+</td>
</tr>
<tr>
<td>175</td>
<td>+</td>
</tr>
<tr>
<td>216</td>
<td>+</td>
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<tr>
<td>114</td>
<td>+</td>
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<tr>
<td>74</td>
<td>+</td>
</tr>
<tr>
<td>249</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>+</td>
</tr>
</tbody>
</table>

S = Suspect  
+ = Reactor  
- = Negative  
(WD) = Born weak and died
TABLE 3
HERD "A"

COMPARISON OF ELISA AND ACF SERODIAGNOSTIC TESTS TO PREDICT BRUCELLA ABORTUS IN REACTOR HEIFERS PRIOR TO PARTURITION

<table>
<thead>
<tr>
<th>HEIFER ID #</th>
<th>TEST RESULTS AT TIME OF REMOVAL OR ISOLATION</th>
<th>CALVING STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACF ELISA</td>
<td></td>
</tr>
<tr>
<td>181</td>
<td>- S</td>
<td>36 days to P</td>
</tr>
<tr>
<td>197</td>
<td>- S</td>
<td>66 days to P (A)</td>
</tr>
<tr>
<td>154</td>
<td>- S</td>
<td>40 days to P</td>
</tr>
<tr>
<td>36</td>
<td>- S</td>
<td>38 days to P (A)</td>
</tr>
<tr>
<td>73</td>
<td>- S</td>
<td>41 days to P</td>
</tr>
<tr>
<td>141</td>
<td>- S</td>
<td>38 days to P</td>
</tr>
<tr>
<td>23</td>
<td>- S</td>
<td>12 days to P (D)</td>
</tr>
<tr>
<td>215</td>
<td>- S</td>
<td>3 days to P</td>
</tr>
<tr>
<td>268</td>
<td>- S</td>
<td>80 days to P</td>
</tr>
<tr>
<td>221</td>
<td>- S</td>
<td>81 days to P</td>
</tr>
<tr>
<td>243</td>
<td>- S</td>
<td>53 days to P</td>
</tr>
<tr>
<td>186</td>
<td>- S</td>
<td>36 days to P</td>
</tr>
<tr>
<td>187</td>
<td>- S</td>
<td>46 days to P</td>
</tr>
<tr>
<td>200</td>
<td>- S</td>
<td>110 days to P</td>
</tr>
<tr>
<td>230</td>
<td>- S</td>
<td>6 days to P</td>
</tr>
<tr>
<td>252</td>
<td>+ S</td>
<td>8 days after P</td>
</tr>
<tr>
<td>199*</td>
<td>+ S</td>
<td>42 days after P</td>
</tr>
</tbody>
</table>

* = 48 days between tests  
(A) = Aborted;  
(D) = Dead  
+ = Reactor  
- = Negative  
S = Suspect  
P = Parturition
### Table 4

**Summary of Results of Testing Herd "B" Through 20 Months**

<table>
<thead>
<tr>
<th>DAY #</th>
<th>MAIN HERD</th>
<th>REACTORS FOUND IN MAIN HERD</th>
<th>ELISA SUSPECT HERD</th>
<th>REACTORS FOUND IN SUSPECT HERD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>104</td>
<td>1</td>
<td>4</td>
<td>----</td>
</tr>
<tr>
<td>210</td>
<td>95</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>270</td>
<td>104</td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>330</td>
<td>102</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>390</td>
<td>102</td>
<td>0</td>
<td>1</td>
<td>1(A)</td>
</tr>
<tr>
<td>600</td>
<td>101</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(A) = aborted

### Table 5

**Summary of Results of Testing Herd "C" Through 24 Months**

<table>
<thead>
<tr>
<th>DAY #</th>
<th>MAIN HERD</th>
<th>REACTORS FOUND IN MAIN HERD</th>
<th>ELISA SUSPECT HERD</th>
<th>REACTORS FOUND IN SUSPECT HERD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>160</td>
<td>0</td>
<td>11</td>
<td>---</td>
</tr>
<tr>
<td>210</td>
<td>160</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>390</td>
<td>160</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>450</td>
<td>156</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>720</td>
<td>156</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>
Fiscal Year (FY) 1985 was a very significant year in the brucellosis program: all parameters measuring progress showed gains; the congressionally mandated State classification goals were met; and a plan for completing the cooperative State-Federal effort was initiated.

The program advancements during FY 1985 were a continuation of the accelerated progress that started 3 to 4 years ago. The two most significant measurements of progress — the numbers of infected herds and the market cattle reactor rate — both declined, while the number of calves vaccinated exceeded the previous year’s total for the eighth straight year. The declining infection rate and other factors made additional funds available for depopulating infected herds. In March, the Deputy Administrator of Veterinary Services called on all personnel to take advantage of this unique opportunity by offering all eligible owners of infected herds the option of depopulation during the remainder of the fiscal year. As a result, 39 percent of all owners of herds eligible for depopulation during the year exercised that option. Excluded from this percentage are the five States (Florida, Louisiana, Arkansas, Texas, and Oklahoma) where depopulation is not practiced or only rarely used. This extensive use of depopulation in FY 1985 effectively eliminated potential spread of the disease from those herds to other herds and areas. Consequently, the effect of this year’s extensive effort will provide a continuing benefit to the program for years to come.

Early in the year, the Administrator of the Animal and Plant Health Inspection Service defined the role the Federal Government will play in the final effort to eradicate brucellosis from the United States. It is anticipated that over a period of approximately 5 years beginning in FY 1986, the traditional cooperative State-Federal effort will change to reduce the role of the Federal Government and to increase the involvement of the States and industry. At the end of that period, the Federal Government will limit itself to those activities which by law or scope cannot be carried out by individual States; specifically, enforcement of interstate regulations, disease surveillance, and the dissemination of information from the program data base at Fort Collins, Colorado.

This phasedown of Federal involvement will require a concerted effort during the next several years to further reduce the level of brucellosis and to increase the number of States that are either free or nearly free of this disease. It will also be a time for each State to examine what this change will mean to it individually and to take whatever steps are necessary to be ready for the added responsibility it will entail. This redirection of responsibility is the logical result of the success of the program which has increasingly changed brucellosis from a national to a regional or in-
vidual State program. This plan will maintain the effectiveness of the cooperative program but increase its efficiency by placing the responsibility for eradicating the last vestiges of infection at the State level where the incentive will be greatest to complete the job.

During FY 1985, the Brucellosis Information System Center (BIS) was renamed the National Center for Animal Health Information Systems. This name more accurately reflects its planned future role as a data base for other VS programs. At the end of the fiscal year, all States except three (Oregon, Oklahoma, and Wisconsin) either were on the system or had made commitment to go on the system. All modules of BIS were completed and are available for implementation by the various States. Use of BIS nearly doubled during the year, and at the end of September there were 12 million tests on the system. As the data base continues to expand, it becomes an increasingly valuable tool for handling the daily management and epidemiological problems of the eradication program.

Also in operation at the end of FY 1985 were the State Regulations Retrieval System and the International Regulations Retrieval System which respectively provide ready access to the current animal import requirements of each State and the import requirements of most foreign countries.

The information on the following visuals is estimated since data for the final month of the fiscal year was not available at the time they were prepared.

Slide 1

On September 30, 1985, 21 States, plus the Virgin Islands, held Class Free status; 19 States, plus Puerto Rico, were class A; 6 States were Class B; and 4 States Class C. In addition, 2 States, Florida and Texas, have both Class B and Class C areas. Minnesota, Montana, and Wyoming qualified for Class Free status during the year; Georgia, Kansas, and Tennessee attained Class A; and Mississippi advanced to Class B, thereby becoming the first Class C State to improve its classification.

Slide 2

There were 6,981 infected herds found in FY 1985, 17 percent fewer than the 8,468 found last year. The majority of this decrease can be attributed to improvement of the brucellosis situation in the high incidence States. Class C States account for 4,169 infected herds, Class B States 2,208, Class A States 600, and the Class Free States had 4 reactor herds, each due to strain 19 infection.

Slide 3

The distribution of infection remains similar to previous years, with 88.1 percent of the Nation's infection occurring in 8 States and 11.9 percent in the rest of the country. There are 34 States, each with less than 30 infected herds, accounting for 1.8 percent of the total. Eight States, having between 30 and 300 each, making up 10.1 percent and 7 States, with 300 to
1,000 infected herds, representing 52.8 percent of the total. Texas had 35.3 percent of the 6,982 infected herds found during the year.

Slide 4
Infection was disclosed in 147 dairy herds as a result of testing brucellosis ring test suspicious herds. There were 2,206 suspicious ring tests of which 1,257 were blood tested.

Slide 5
Thirteen and nine-tenths million cattle were tested under the Market Cattle Identification (MCI) program in FY 1985, a decrease of 800,000 from the 14.7 million tested last year. Of these, 43.7 percent were tested at packing plants and 56.3 percent at other places. The decline in the MCI tests in FY 1985 reflects a reduction in slaughter from last year when drought conditions in certain areas caused increased culling in many herds.

Slide 6
The total number of cattle tested in FY 1985 was 19.1 million, with 5.2 million of these tested on farm or ranch and 13.9 million tested under the MCI program. The number of reactors found declined from 125,000 in FY 1984 to 105,000. Although there was a 6 percent decline in total cattle tested in FY 1985, there was a 16 percent decline in the number of reactors found.

Slide 7
The number of calves vaccinated reached 9.6 million in FY 1985, an increase of 800,000 over FY 1984. This is the eighth year in a row that the number of calves vaccinated exceeded the total for the preceding year.

Slide 8
The number of swine tested for brucellosis in FY 1985 was 2.9 million, slightly higher than the number tested in FY 1984. This total included 2.4 million tested under the Market Swine Testing (MST) program and 447,000 tested on farms.

Slide 9
The reactor rate on all tests increased from 0.39 percent in FY 1984 to 0.043 percent in FY 1985. The rate of on-farm reactors more than doubled, from 0.057 percent to 0.12 percent, over the same period. This indicates that infection on the farm, though low, was more advanced when first diagnosed and that more infected swine herds were retested this year rather than depopulated. The MST reactor rate, the best indicator of the national prevalence of swine brucellosis, declined from 0.36 percent in FY 84 to 0.029 percent this year.
Slide 10

One State, New York, attained Validated Brucellosis-Free area status — Stage III — during the year, bringing to 27 the number of States that have achieved this goal. The majority of the Nation’s swine are now located in States that are validated Brucellosis-Free.


Seven States, Alabama, Arkansas, Connecticut, Georgia, Hawaii, Louisiana, and Virginia, were in Stage II. Eleven States, Florida, Kansas, Kentucky, Massachusetts, Michigan, Nebraska, New Jersey, North Carolina, Ohio, Oklahoma, and South Carolina, were in Stage I. Seven States, Mississippi, Missouri, New Mexico, Oregon, Tennessee, Texas, and West Virginia, remained in the “no program” classification at the end of the year.

Slide 11

The number of Validated Brucellosis-Free herds increased from 3,171 in FY 1984 to 4,184 at the end of this year. This 24 percent increase partially offsets the significant reduction that occurred in 1984 from the 4,558 validated herds reported the previous year.

The incidence of swine brucellosis in the United States is extremely low; the main obstacle to the early eradication of this disease continues to be the lack of adequate identification for tracing MST reactors.
Cattle Brucellosis

State Classifications

<table>
<thead>
<tr>
<th>Number</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.3%</th>
<th>0.3%</th>
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<tbody>
<tr>
<td>Free</td>
<td>21</td>
<td>19</td>
<td>6</td>
<td>2</td>
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<tr>
<td>Class A</td>
<td></td>
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<tr>
<td>Class B</td>
<td></td>
<td></td>
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<tr>
<td>Class C</td>
<td></td>
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</tbody>
</table>

*Not included:
States with dual status:
Florida and Texas—B and C
District of Columbia—Free
Virgin Islands—Free
Puerto Rico—A
Yellowstone National Park, WY—Not Classified

September 1985
Swine Brucellosis

Validated Herds FY 1985

*Estimated.
Brucellosis Eradication

Percent of Total Reactor Herds Found

*Fiscal Year 1985
Total Herds: 6,981

35.3%
States: 1
Herds: > 1,000
Total Reactor Herds = 2,467

1.8%
States: 34
Herds: < 30
Total Reactor Herds = 124

10.1%
States: 8
Herds: 30 < 300
Total Reactor Herds = 702

52.8%
States: 7
Herds: 300 < 1,000
Total Reactor Herds = 3,688

*Estimated.
Brucellosis Eradication

Milk Ring Test Results (BRT)

- Total Suspicious BRT Tests
- Follow-up Herd Blood Tests
- Infected Herds Found

Fiscal Year

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<tr>
<td>Total Suspicious BRT Tests</td>
<td>2,179</td>
<td>2,450</td>
<td>2,018</td>
<td>2,586</td>
<td>2,177</td>
<td>3,091</td>
<td>2,553</td>
<td>2,773</td>
<td>3,607</td>
<td>3,519</td>
<td>2,412</td>
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<tr>
<td>Follow-up Herd Blood Tests</td>
<td>2,050</td>
<td>2,012</td>
<td>1,629</td>
<td>1,601</td>
<td>1,544</td>
<td>350</td>
<td>350</td>
<td>353</td>
<td>353</td>
<td>281</td>
<td>215</td>
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<td>Infected Herds Found</td>
<td>392</td>
<td>641</td>
<td>435</td>
<td>350</td>
<td>317</td>
<td>353</td>
<td>260</td>
<td>205</td>
<td>281</td>
<td>281</td>
<td>1,257</td>
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*Estimated.
Brucellosis Eradication
Market Cattle Testing Program

Fiscal Year | At Packing Plants | Other
--- | --- | ---
1975 | 70.0% | 30.0%
1976 | 69.6% | 30.4%
1977 | 67.5% | 32.5%
1978 | 62.2% | 37.8%
1979 | 54.8% | 45.2%
1980 | 41.7% | 58.3%
1981 | 42.0% | 58.0%
1982 | 44.4% | 55.6%
1983 | 43.3% | 56.7%
1984 | 45.6% | 54.4%
*1985 | 43.7% | 56.3%

*Estimated.

Millions of Cows Blood Tested
Brucellosis Eradication

Blood Testing: Cattle

- Farm or Ranch
- MCT

Millions Cattle Tested

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<td>2000</td>
<td>17.7</td>
<td>20.8</td>
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<td>19.5</td>
<td>19.4</td>
<td>19.4</td>
<td>20.3</td>
<td>19.1</td>
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Thous Reactors Found

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<tr>
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<td>250</td>
<td>236</td>
<td>241</td>
<td>197</td>
<td>195</td>
<td>193</td>
<td>176</td>
<td>154</td>
<td>125</td>
<td>105</td>
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</table>

*Estimated.
Brucellosis Eradication

Calves Vaccinated

Fiscal Year

*Estimated
Swine Brucellosis

Animals Blood Tested

Thous. Animals

Fiscal Year


*Estimated
Swine Brucellosis

Infection Rate

Percent

0.5


Fiscal Year

Total Tests  On Farm  MST

*Estimated
Swine Brucellosis

Program Stages—Sept. 30, 1985

Stage 1
Stage 2
No Program
Stage 3
(Validated-Free)
Brucellosis Eradication

Number of Infected Herds Found
(According to State Classification)

State Classification
- Certified-Free
- Modified Certified
- Noncertified

New State Classification (Effective May 1, 1982)
- Class Free
- Class A
- Class B
- Class C

Thousands
- 20

Fiscal Year
- '78
- '79
- '80
- '81
- '82
- '83
- '84
- '85

Table:
<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Certified-Free</th>
<th>Modified Certified</th>
<th>Non-Certified</th>
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<tr>
<td>1978</td>
<td>27</td>
<td>23</td>
<td>0</td>
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<tr>
<td>1979</td>
<td>30</td>
<td>20</td>
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<tr>
<td>1980</td>
<td>31</td>
<td>19</td>
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</tr>
<tr>
<td>1981</td>
<td>32</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>1982</td>
<td>32</td>
<td>18</td>
<td>0</td>
</tr>
</tbody>
</table>

New Classification
- States with dual status:
  - Montana-Class Free and Class A
  - Texas and Florida-Class B and Class C

*Estimated.
Mr. John Armstrong opened the Brucellosis meeting at 1:45 p.m. with welcoming remarks. He asked that the agenda and time table be upheld due to the limited time allotted and amount of material needed to be covered. He stressed the need to keep private veterinary practitioners involved and interested in the Brucellosis Control and eradication effort and to recognize the cooperation of the producers in this area.

1. Dr. Billy Johnson presented the report on the National Brucellosis Program. There are 21 Class Free States, 19 Class A states plus Puerto Rico, 6 Class B and 4 Class C states. The market cattle reactor rate continued to decline to a final rate of 0.26% for the year. All states are now using reduced dosage Strain 19 vaccine. The Brucellosis Information System moved forward rapidly this year with all except three states being on the system of committed to enter it. Depopulation funds were made available to all except five higher incidence states.

2. Dr. Granville Frye presented the results comparing the modified card test with those of rivanol and complement fixation (CF) test. He recommended that this modified card test not be approved for use in livestock markets as it is felt that there are too many false negative results based on the rivanol and CF tests as the standards. Dr. Frye also gave an update on the rewriting of the Uniform Methods and
BRUCELLOSIS

Rules (UM&R). Committee members were asked to critically review the rewritten version.

3. Dr. Fred McCrory presented a resolution submitted by the Southern Animal Health Association concerning retattooing of official calfhood brucellosis vaccinates. He requested the Brucellosis Committee to consider authorizing the retattooing of official vaccinates under the following criteria:
   a) Identification of vaccinated animal(s) can be verified by official records maintained in state or federal office.
   b) Prior approval for retattooing must be obtained by either the State Veterinarian or the Area Veterinarian in Charge.
   c) Retattooing will produce the original tattoo at time of vaccination.

4. Dr. John Cobb discussed mandatory first point testing in “B” and “C” areas or states and submitted a motion requiring a brucellosis test on all test eligible cattle in markets, buying stations or assembly points used for sale or trade of livestock.

5. Dr. Joe Hendricks gave a report on the complement fixation review and listed two recommendations to the committee:
   a) Those states which are using the same CF method could produce more similar results if a larger bank of reference serums were available. (NVSL could provide the service.)
   b) The development of new CF methods should be encouraged but should be approved by the USAHA Brucellosis Committee before utilized as official tests.

6. Mr. Jack Dahl reported from the subcommittee for movement of heifers from quarantined herds. The subcommittee felt that a better approach to the problem of the heifer syndrome at this time could be achieved by recommending program changes that would accelerate the identification of herds of unknown status in the “B” and “C” states. Changes for the UM&R were mailed earlier and action was requested on these proposed changes.

Dr. James Alexander reported on evaluation standards for C and B states.

7. Dr. Lee Alley’s report on current problems relating to “direct movement” of cattle for slaughter will be presented October 29, 1985.

8. Dr. Garry Adams reported on the subcommittee for Buffered Acidified Plate Antigen (BAPA) and Rapid Screening tests. The subcommittee recommends that the modified card test should not be approved for use in livestock markets due to excessive false negative results; and that rather than delete the entire paragraph of Chapter 1, UM&R, only delete “or herds with presumptive evidence of infection (suspicious brucellosis ring test, market cattle identification reactor, contact herd, epidemiological traces, etc.). It was also their
opinion that the RST be phased out as a presumptive test for classification of negative cattle because of high titer prozone reactions which cause an excessive number of false negative results.

9. Dr. W. Clark presented a paper on the “Movement of Yellowstone Park Brucellosis Infected and Exposed Bison.” He described the history of the bison in the Yellowstone area and the “boundary control” program used in Yellowstone. Seasonal movements and trails were explained.

10. The report on “Further Studies on Adult Vaccination of Cattle in Brucellosis Negative Herds” by Dr. Joe Hendricks and Dr. John R. Lomme was cancelled. The report will be given next year.

11. Dr. Drazek gave a few New York experiences using the reduced dose of Strain 19 vaccine. He stressed that vaccination is only one tool used, and overreliance on the vaccine should be avoided.

12. Drs. Bechtol and Reed presented new concepts in brucellosis vaccinations. Their proposal of a ballistic vaccination system using a “tagged” Strain 19 vaccine would eliminate catching and identifying animals (for use in wild populations), would possibly reach the 80% needed for control, and would differentiate between vaccinated and infected animals.

13. Mr. Alfred Keating reported on the Livestock Conservation Institute meetings that were held in July. The purpose of the meetings was to attempt to spur progress toward achieving free status in the class “A” and “B” states. Based on the goals set by state officials attending the meetings, by the end of 1988 there will be 36 states plus the Virgin Islands free of bovine brucellosis. An additional 9 states will be in class “A”. Those 36 states expected to be free by the end of 1988 have 48% of the herds and 52% of the cows in the country.

14. Jim Horne submitted a report on the Education subcommittee. He requested that the Brucellosis Committee adopt the recommendations made by the committee.

15. Bill Bulmer reported on Canadian results. Canada is now classified as being free; but they are still using the full dosage of Strain 19.

The meeting was recessed until 1:30 p.m. October 29, 1985.

USAHA BRUCELLOSIS COMMITTEE

Minutes—Tuesday, October 29, 1985

The meeting was called to order by the Chairman, Mr. John Armstrong, at 1:45 PM.

Dr. Paul Doby presented the Swine Brucellosis Subcommittee report. The complete report is given as Attachment 1 following the Brucellosis Committee minutes. It was moved and seconded that the report be accepted. The motion carried.

The agenda items that were discussed on October 28, 1985, were then
presented for discussion and further action. They are listed with their agenda item number from the previous day's meeting for easier reference. The adopted motions are included as attachments following the minutes.

7. Current problems relating to "direct movement" of cattle for slaughter

A subcommittee consisting of Dr. Lee Alley, Dr. Granville Frye, and Mr. George Hall reviewed the problems experienced by states with low incidence of brucellosis and no major slaughter facilities with regard to the Office of General Counsel's interpretation of "direct movement." The subcommittee's report was made by Dr. Lee Alley and recommended that cattle be allowed to move through one approved concentration point during shipment to slaughter or a quarantined feedlot as a direct movement.

After a minority statement by Dr. Frye concerning the intent of the current requirements, the committee moved to adopt the subcommittee report as presented by Dr. Alley. It was seconded and passed. The report and Dr. Frye's statement are Attachments 2 and 3.

12. New concepts in brucellosis vaccination

Mr. Jack Dahl moved that the committee adopt the program presented by Drs. Bechtol and Reed and establish a subcommittee to provide guidance in an evaluation study with a report to be made at the USAHA annual meeting in 1986 and to encourage the industry and USDA to support this new concept.

Dr. Bechtol then gave a short demonstration of the vaccine delivery system and commented on the use of the system with Strain 19 vaccine in Wyoming.

This motion was seconded and passed. The report by Drs. Bechtol and Reed and Mr. Dahl's motion are Attachments 4 and 5, respectively.

1. Increase indemnity rates for herd depopulation

Dr. Granville Frye presented a motion to amend Title 9 CFR to increase indemnity rates for herd depopulation. Dr. Hudelson received assurance that movement brand laws would satisfy the dealer recordkeeping provision. Mr. Dahl recommended an amendment limiting depopulation payment to only once per herd owner. After discussion concerning the possible problems this could cause, this amendment was moved, seconded, but not passed.

The original recommendation (Attachment 6) was moved, seconded and passed.

8. Modified card test

Dr. Granville Frye presented the Scientific Advisory Committee's recommendation that the modified card test not be approved for use
REPORT OF THE COMMITTEE

in the markets due to problems encountered with sensitivity and specificity.

It was moved and seconded that the report be adopted. The motion carried. (Attachment 7)

Dr. J. F. Badger recommended that work should continue to develop a test that would help alleviate the problems encountered with vaccination titers at the market.

3. Retattooing of heifers

Dr. McCrory moved that the committee adopt the Southern Animal Health resolution on establishing a national policy for retattooing of heifers. It was seconded.

Dr. Vanderwagen presented the proposed amendment by the Western States Association to delete reference to the Area Veterinarian in Charge since vaccination is regulated by state laws. Dr. McCrory accepted the amendment. Dr. Johnson moved that the amendment not be made. This was seconded but did not pass. The original motion was passed as amended by Dr. Vanderwagen. (Attachment 8)

4. Mandatory first point of concentration testing in Class B and C areas or states

Dr. John Cobb moved that the UM&R require that first point of concentration testing of all test eligible cattle be mandatory in Class B and C areas or states. This was seconded by Mr. John Adams, but the difference in target dates was questioned.

Mr. George Hall representing the Oklahoma City and St. Louis Stockyards objected to the cost to the markets and industry due to extra handling of livestock.

Dr. Badger recommended that “test eligible cattle entering quarantined feedlots must be tested.” This was accepted by Dr. Cobb and Mr. Adams.

Mr. John Cargile objected to Dr. Badger’s proposal with Dr. Hartin’s concurrence.

Dr. Becton recommended that the wording be such that cull cows from an established herd of origin would be tested, and not put together heifers.

The Chairman recommended the committee table the proposal so Dr. Becton could prepare the correct wording for an amendment. This was moved, seconded and passed. The motion was tabled. (Attachment 9) This motion remained tabled due to action taken in the report on the movement of heifers from infected herds.

5. Review of Complement-Fixation Tests

The committee moved to accept the report by Dr. Joe Hendricks for the subcommittee for review of the Complement-Fixation Test. This was seconded and passed. (Attachment 10)
6. Movement of heifers from quarantined herds

Mr. Jack Dahl reminded the committee of Dr. Berman’s warning that identifying the heifers from infected herds will give a false sense of security. He then presented the report of the subcommittee on the movement of heifers from infected herds and moved that it be accepted. Dr. Espe seconded it.

Mr. Bill Gallagher indicated the NCA subcommittee had an amendment which was to be presented by Mr. John Cargile. After brief comments on the progress being made in Texas, Mr. Cargile explained the amendment to the Termination Date section and moved that it be adopted. This was seconded and carried.

Dr. Badger proposed an amendment to delete Option 2 and modify Option 1 to refer to beef heifers and substitute “‘F’ brand” for “‘S’ brand.” This was seconded. After much discussion regarding these amendments and changes in vaccination ages, the amendment failed.

Following the discussions from the floor regarding the need for mandatory whole herd vaccination and the changing of vaccination ages, a motion was made to amend Option 2 and 3 to provide for an approved herd plan in which whole herd vaccination will be included and the ages for calfhood vaccination would be reduced when recommended by an epidemiologist. This was seconded and passed.

A motion was made by Dr. Bob Hartin to amend the original motion by Mr. Dahl so the committee report could be considered in separate parts. It was seconded but failed to carry.

Dr. Gregg Nelson moved that item 3 in the Additional UM&R changes referring to a reduction in the maximum calfhood vaccination age be deleted. This was seconded and carried.

Mr. George Hall moved that reference to selling restricted heifers through markets be added to the Restricted Calves for Grazing Section. This was seconded, but Mr. Dahl moved a substitute motion that the entire section be deleted and a subcommittee be appointed to study this concept and report back next year. This was seconded and carried.

Dr. John Cobb moved that Item 1 of the Additional UM&R changes be amended to add “a test is required on all past parturient cows entering quarantined feedlots.” This seconded and carried.

Mr. Dahl’s original motion was adopted with the approved amendments. (Attachment 11)

8. Use of Buffered Acidified Plate Antigen (BAPA) and Rapid Screening Test (RST) for classifying cattle as negative

The committee voted to adopt the Scientific Advisory Committee’s recommendation on the use of the BAPA and the RST for classifying cattle as negative as recommended by Dr. Joe Rinehart. (Attachment 12)
14. Education Subcommittee Report

The committee voted to adopt the Education Subcommittee report as presented by Mr. Jim Horne. (Attachment 13)

Mr. J. O. Pearce moved that the UM&R be modified in Part II, Section R in reference to the time frame for retesting adult vaccinated certified free herds. This was seconded and passed. (Attachment 14)

Mr. Al Keating urged the allied organizations to support the House version of the brucellosis appropriation measure.

Mr. Armstrong introduced Dr. Frank Mulhern for closing comments. He urged everyone not to get discouraged because progress is difficult. He said progress is being made, but care must be taken to ensure that modifications do not make the program ineffective as has happened in the past. He stressed optimism, leadership and setting of goals and to get the government out of the brucellosis business by eradicating the disease.

The meeting adjourned at 5:35 PM.

REPORT OF THE SUBCOMMITTEE ON SWINE BRUCELLOSIS

Paul B. Doby, Chairman

The meeting was called to order at 1:30 p.m., October 28, by Chairman Paul Doby with the following members present: Neal Black, Jim Quigly representing John Cobb, Alfred M. Creswell, Mitchell Essey, Robert E. Hall, Merle H. Lang, David Meisinger, Phillip Pickerill and C. Donald Van Houweling.

Dr. Victor F. Nettles of the Committee on Wildlife Diseases, opened the meeting with new information regarding swine brucellosis in feral swine. He stated that eight states are known to have feral swine positive with brucellosis with the addition of Texas to the list during the past year. A total of nineteen (19) states have feral swine.

Following a presentation by Chairman Doby to the annual meeting of the International Association of Fish and Wildlife Agencies at Sun Valley, Idaho in September regarding brucellosis in feral swine, Jack H. Berryman, executive vice-president of the organization sent a memorandum to all governmental members on the roles of the organization, encouraging the members to communicate and work with animal health offices to: 1) stop indiscriminate relocations of wild swine of unknown disease status, and; 2) minimize future contact of infected wild swine with domestic swine. Dr. Nettles distributed a list of the officials of fish and wildlife agencies in states with wild swine populations as an aid in stimulating communications between those officials and livestock disease regulatory officials. Dr. Nettles suggested that lists of sources for negative feral swine be developed and circulated to hunting preserves and wildlife departments in states with hunting preserves. He also stated that states need improved
regulations in respect to intrastate movement of feral swine. In response to a question, it was indicated that laboratories can differentiate their test result data between feral and domestic swine. It was pointed out that some wildlife agencies would like to eliminate feral swine from state forests and other public lands and such swine, if tested negative for brucellosis, might become a source of brucellosis-free animals for hunting preserves.

Reports from states with feral swine presented included:

**Georgia** — Reported that in FY’85 they had 39,965 slaughter swine samples with no reactors, while 8,472 sows and boars which could have gone back to farms, were tested at markets where 12 reactors were disclosed.

**Oklahoma** — Their testing requirements for movement include feral swine. A change in their ownership testing requirements include feral swine. Their problem is that the 5,000 to 6,000 feral swine in their state are concentrated in the southwesternmost county which is the greatest problem in terms of testing for bovine brucellosis. Hogs in that area are being trapped and smuggled out-of-state, therefore, no test data is available to indicate the brucellosis status of these hogs.

**N. Carolina** — Feral swine in the Smokey Mountain area of N. Carolina are not known to be positive. All test results on these hogs have been negative.

**Arizona** — There are only a few swine in Arizona and wildlife authorities would like to eliminate them, so it is possible that in a short time their numbers will be reduced. There is no evidence that they are infected.

Dr. Mitchell Essey of APHIS reported on the status of swine brucellosis eradication during fiscal year ’85 as follows:

There are twenty-seven (27) validated states and only seven (7) reported states with swine brucellosis during the past year, indicating that the time is ripe for a push to wipe out the disease.

Infected herds by state were disclosed as follows:

<table>
<thead>
<tr>
<th>State</th>
<th>Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>20</td>
</tr>
<tr>
<td>Alabama</td>
<td>13</td>
</tr>
<tr>
<td>New Jersey</td>
<td>6</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>5</td>
</tr>
<tr>
<td>Florida</td>
<td>7</td>
</tr>
<tr>
<td>South Carolina</td>
<td>1</td>
</tr>
<tr>
<td>Hawaii</td>
<td>1</td>
</tr>
</tbody>
</table>

2.9 million swine were sampled during the fiscal year. 2.4 million were in the market swine testing program and nearly half a million on farms. He said that 2.2 million sows and boars tested at slaughter represented 38.5% of the 5.7 million sows and boars slaughtered during the year.
Sixty-four percent (64%) of the slaughter samples were collected by contract bleeders, seven percent (7%) by meat inspection personnel, twenty-four percent (24%) by packer employees and 4.4% at 3,149 state inspected establishments. Cost of the samplings ranged from .25¢ to .32¢ per sample on a contract basis.

The positive reactor rate, based on confirmation of the card test by supplemental tests, increased in FY'85 to .043% from .039% the previous year, with the increase coming from on-farm testing.

In the market swine testing program, 53% of the sow and boar samples were identified, but Dr. Essey noted that in some plants, only swine known to be identified are sampled, biasing that figure.

Of the card test positives, twenty percent (20%) were Rivanol positive. In terms of cases closed during the fiscal year, there were 167 Rivanol positives and 477 card positive, Rivanol negatives, of which 311 were successfully traced, 100 were traced to other states and 233 could not be traced, giving a successful traceback rate of fifty-seven (57%).

Dr. Essey was asked to provide data to the committee on the rate of successful traceback of just the Rivanol positives and he said he would do so.

The on-farm tests, which include followup testing of market swine positives, revealed 53 infected herds of the 25,092 herds tested, a rate of .2%. The infected swine rate was .12%.

Dr. Essey pointed out that since 1980, the annual total number of new infected herds disclosed has dropped from 192 to 53 and the number of states with infected herds from 21 to 7 for the past two years.

The validated herd total is 4,184 which represents a recovery of much of the loss from 1984. The number of validated herds has remained at 4,000 to 4,500 for a number of years.

APHIS is considering development of a program to complete swine brucellosis eradication by 1990. The subcommittee was asked to provide guidance in developing a plan to accomplish that objective. The subcommittee will assemble ideas during the next few months by mail and will meet to develop a final recommendation during the annual meeting of the Livestock Conservation Institute in Omaha next April.

The subcommittee deferred action on republication of an indemnity increase to conform with the 5-year plan being developed.

Ms. Mabel Owen of Massachusetts reported that her state is seeking an alternative means of revalidation other than the herd testing used in validation, since personnel is not available for rebleeding. One alternative is slaughter sampling, since there is good cooperation from small slaughter plants in the state.

Neal Black discussed a proposal for first-point testing as an alternative to slaughter testing. He suggested such a system might be especially
valuable in pseudorabies monitoring, but might also be studied for its application for brucellosis and trichinosis surveillance.

Mr. Black moved, seconded by Hall, that the subcommittee endorse amendments to the CFR with regard to swine brucellosis regulations as published in the Federal Register, Volume 50, Number 177, dated September 12, 1985. It was carried.

Chairman Doby reported on a survey of the seven states with no swine brucellosis eradication programs.

Missouri — Dr. Ed Slauter said plans are being developed to implement an eradication program. An advisory committee is being established which will be meeting in January 1986.

The states of Texas, Mississippi, Oregon, West Virginia, Tennessee and New Mexico did not present any plan to initiate a swine brucellosis program.

There being no further business, the meeting was adjourned.

REPORT OF THE SUBCOMMITTEE ON DIRECT MOVEMENT

Dr. Lee Alley

Brucellosis restricted (B & S brands) cattle be allowed to move from the livestock market through one approved concentration point for shipment to slaughter or to a quarantine feedlot. The restricted cattle would be handled as restricted cattle at the concentration point. Non-restricted slaughter cattle could also move through the concentration point for shipment to slaughter. Restricted cattle moving under seal would not be allowed to be off-loaded through a concentration point.

MINORITY STATEMENT OF THE DIRECT MOVEMENT SUBCOMMITTEE

Dr. G. H. Frye

The movement requirements in Part 78 are for the purpose of minimizing the spread of brucellosis by animals moved interstate.

Since known infected and exposed animals present the greatest risk of spreading the disease these animals must be handled in a manner that is commensurate with the risk involved. The safest way would be for Rx and exposed animals to move directly from the farm of origin to a slaughter plant without any intermediate stops.

However, to facilitate movement, such animals are permitted to move to one approved market enroute to slaughter. This is in accordance with previous recommendations from this committee.

What is proposed now is that after reactors and exposed animals have been moved to an approved market, a second movement would be per-
mitted into concentration points that may or may not be handling other than slaughter animals.

The risk of exposure to brucellosis increased with every addition movement. At this point in the Eradications Program we believe the risk of exposure should be reduced rather than increased.

The U.S. position is the one expressed in the current CFR and the one that is now out for comment. That is that reactor and exposed animals should move directly from an auction market to slaughter. However, the purpose of publishing amendments for comment is to obtain industry input. The wishes of this committee, whatever it may be, will be considered at the end of the comment period.

NEW CONCEPTS IN BRUCELLOSIS VACCINATION

D. Bechtol and D. Reed
BallistiVet, Inc. White Bear Lake, Minnesota
and Molecular Genetics, Inc., Minnetonka, Minnesota

This report is: 1) to advise the Committee of certain technologic advances which can aid in the control of Brucellosis, 2) to urge adoption of control policies which make use of these advances, and 3) to ask the Committee's support for funding and implementing an improved vaccine for Brucellosis.

The technologies developed by BallistiVet and Molecular Genetics are:

1. A ballistic vaccination system capable of delivering vaccine-containing biodegradable bullets from a compressed air rifle. The bullets can be delivered up to 60 feet, penetrate 2-3 cm, and dissolve within 10 hours to release the vaccine.

2. Recombinant DNA methods for modifying the Strain 19 vaccine to (a) add the gene for a specific new antigen as a unique serologic tag to identify vaccinates and (b) to delete a specific Strain 19 Brucella gene and to use the antigen specified by that gene as the basis of a serologic test to distinguish infected animals from vaccinates.

In order for any Brucella vaccination program to be successful it must extend to at least 80% of the “at risk” population. It is our proposal to develop a Brucella vaccine with a built-in tag and to develop compatible serologic tests which can identify vaccinates and distinguish vaccine response from serologic response to field infection. The serologic tag could replace the current tattoo and vaccination tag and, in conjunction with a ballistic delivery system, would make it practical to vaccinate free-living wildlife and unconfined cattle. Ear tagging, in our proposal, would be required only upon sale or shipment of the cattle. Two serologic tests would be required at shipment or sale: a test to determine vaccination status (detects serologic response to the unique antigenic tag) and a test to determine infection status (either the currently used tests or an improved test which would detect serologic response to an antigen genetically deleted from Strain 19 but present in virulent Brucella).
What we propose has many features of the ideal vaccine. That is: a vaccine delivery system which does not require catching and identifying animals and companion diagnostics which are used to identify vaccinates and distinguish vaccinates from infected animals.

MOTION TO ADOPT REPORT ON NEW VACCINATION CONCEPTS

Jack Dahl

Move that this committee adopt the program (as described by Drs. Bechtol and Reed) for the development of a serologically tagged Brucella Vaccine, suitable for administration via conventional or ballistic methods, which can be identified by a rapid test method specific to this tagged antigen. Further, that the USAHA Brucellosis Committee encourage this program by establishing an advisory sub-committee to guide BallistiVet and Molecular Genetics Inc. (MGI) through an evaluation study, focused on determining the efficacy of this system during the next twelve (12) months, and every effort be made to encourage USDA and industry to financially support this new concept opportunity.

A progress report shall be presented at the USAHA 1986 annual meeting.

RECOMMENDATION TO INCREASE INDEMNITY RATES FOR DEPOPULATION

That APHIS, VS, amend Title 9, CFR, Part 51, to increase indemnity rates for herd depopulations as an incentive for owners to depopulate their herds. Rates to be established by the Deputy Administrator based on available funds but at levels that do not make brucellosis profitable to owners of infected herds.

Further, that the new rates are to be based on state classification with those in free states based on appraisals with flat rates in all other classifications which become progressively lower for Class A, Class B, and Class C states to provide an incentive to move up in the program status. Indemnity payments shall be at the rates in effect at the time the herd is declared infected or when the owner is first offered depopulation. These increased rates will be available to states effectively carrying out all aspects of the Brucellosis eradication program including:

1. Adequate import laws
2. Adjacent and contact herd testing
3. Epidemiological investigations of all infected herds
4. Dealer licensing laws

BRUCELLOSIS SCIENTIFIC ADVISORY COMMITTEE

Use of Modified Card Test in Lieu of Rivanol Test for Markets

Dr. G. H. Frye

It is the opinion of the Brucellosis Scientific Advisory Committee that
based upon the 8181 modified card test results obtained from ten (10) states from cattle vaccinated as calves or adults and compared to the rivanol and complement fixation tests, the modified card test should not be approved for use in livestock markets due to excessive false negative results (i.e., excessively low sensitivity — 64.1% for calfhood vaccinates and 66.9% for adult vaccinates).

SOUTHERN ANIMAL HEALTH ASSOCIATION
RESOLUTION
Retattooing of Heifers

WHEREAS, the Code of Federal Regulations, Part 78.1 requires the permanent identification of official vaccinates to include a legible official calfhood vaccination tattoo in the right ear.

WHEREAS, the ink used in applying a tattoo has in fact had durability problems to the point of not being legible over a period of time.

WHEREAS, herd management and the sale and movement of vaccinates both intrastate and interstate is dependent upon a legible tattoo being in the right ear.

The State Veterinarian membership of the Southern Animal Health Association requests the Brucellosis Committee to consider authorizing the retattooing of official vaccinates under the following criteria.

1. Identification of vaccinated animal(s) can be verified by official records maintained in state or federal office.

2. Prior approval for retattooing must be obtained from the State Veterinarian.

3. Retattooing will reproduce the original tattoo at time of vaccination.

PROPOSAL FOR MANDATORY FIRST POINT TESTING IN HIGH INCIDENT STATES
John A. Cobb, DVM

1. Good surveillance is the first key to brucellosis eradication.

2. First point testing has proven to be the most effective surveillance tool to date.

3. High incidence states must make great progress if the country is to be in sight of free status within five years.

4. One or more Class B and C states are not presently requiring first point testing at markets and buying stations.

MOTION: Require in the Uniform Methods and Rules for Brucellosis Eradication that all C and B states must perform brucellosis testing on all test eligible cattle in markets, buying stations or assembly points used for sale or trade of livestock.

Require B states to begin this first point testing as of July 1, 1986.

Require C states to begin the first point testing as of January 1, 1987.
BRUCELLOSIS

SUB-COMMITTEE FOR REVIEW OF THE COMPLEMENT-FIXATION TEST

There are 27 states and Puerto Rico which conducted a complement-fixation (CF) test for brucellosis. Twenty-six (26) laboratories utilize the "Wisconsin" method, a cold fixation method, (20 use microtiter plates and 6 use glass tubes). One state uses a Technicon Auto II for an "automated" method and one state conducts a warm fixation test in microtiter plates. Thus 27 of the 28 states are using a complement fixation method approved in the U.M. and R.

Although most laboratories are using the same or similar CF methods, there has been little effort to standardize the results of these laboratories. At the present time, there are three (3) "control" serums available from NVSL. These serums have "low," "medium" and "high" titers to the Wisconsin method. However, a much larger number of serums will be necessary before a more exact standardization between laboratories can be accomplished.

RECOMMENDATIONS

1. Those states which are using the same CF method could produce more similar results if a larger bank of reference serums were available. It is suggested that NVSL provide this service to laboratories upon request.

2. The development of new CF methods should be encouraged. However, before they are utilized as official tests or for the condemnation of animals, they should be approved by the USAHA Brucellosis Committee. The committee's decision should be based on the recommendation of the scientific advisory committee following its review of data relating to that test.

USAHA BRUCELLOSIS SUBCOMMITTEE ON MOVEMENT OF HEIFERS FROM QUARANTINED HERDS

The current procedures in the UM&R Part II M, for moving calves from infected herds would be deleted and replaced with the following:

Option 1. For Class A and Free States — all herds except commercial dairy herds which meet requirements in Option 3.

All heifer calves would be included in the quarantine. Movement would be restricted to those which are "S" branded, "spayed" or held separate and apart from the rest of the herd and remain under quarantine until they pass a negative brucellosis test following the completion of calving. If these heifers remain with the herd, the entire herd shall remain under quarantine until the heifers have completed calving and the entire herd has passed a negative brucellosis test. Calves to be retained in the herd should be calfhood vaccinated. (Restricted calves would move according to 9 CFR 78.8 Brucellosis Exposed Cattle.)

Option 2. For Class C and B States — all herds except commercial dairy herds which meet requirements in Option 3.
a. Herd Plan — the herd must be on schedule following an approved herd plan which will include adult vaccination and reduced age for calfhood vaccination when recommended by an epidemiologist.

b. Mandatory vaccination of all heifer calves — all heifer calves must be vaccinated between the ages provided for in the herd plan.

c. Heifer calves which are eight months of age or less may be sold unrestricted if the above requirements are met. These calves must be vaccinated for brucellosis prior to leaving the premises.

d. States or herds which are unable or unwilling to follow the above requirements would use Option 1 (for A and Free States).

Option 3. Dairy Herds — All states — Commercial dairy herds Calves may move from quarantined dairy herds under the following conditions:

a. Herd Plan — the herd must be on schedule following an approved herd plan which will include adult vaccination and reduced age for calfhood vaccination when recommended by an epidemiologist.

b. Mandatory vaccination of all heifer calves — all heifer calves must be vaccinated between the ages provided for in the herd plan.

c. All calves (male and female) must be identified with an official identification tag prior to movement.

d. They may be no more than six months of age at the time of movement and were separated from the dam at no more than seven days of age.

e. They must be quarantined separate and apart from other cattle for no less than 30 days after movement from the infected herd.

f. Calves of eligible age must be vaccinated for brucellosis prior to movement.

g. Those not meeting the above requirements would follow Option 1 (for Free or A States).

TERMINATION DATE

The proposed movement of calves from infected herds in Class B states be terminated as of October 1, 1988 and furthermore, as of that date, no cattle may be moved from Class C states except steers, spayed heifers, “S” branded cattle or those from certified free herds. As of October 1, 1990, these restrictions will apply to Class B states that are not meeting the standards necessary for progress.

HERD PLAN TO INCLUDE HEIFERS IN INFECTED HERDS

(ALL STATES)

In order to identify and account for heifers in infected herds, the following would be required as part of the herd plan for all infected herds. (Free, A, B or C status).

On the first herd test following disclosures of brucellosis reactors on the farm, all heifer calves four to eight months of age shall be officially vaccinated for brucellosis. In those states where calfhood vaccination in infected herds is not mandatory, and the owner elects not to vaccinate, all
calves in this age group (four to eight months) shall be identified with an official metal ear tag. Those calves over eight months of age shall be considered test eligible.

All calfhood vaccinated heifers which have been vaccinated for more than six months shall be subject to periodic tests. (First tests at 14-16 months of age.)

If the heifers commingle with the adult herd, they shall be tested at the same intervals as the adult cow herd. If they are maintained as a separate group, they should be tested immediately prior to breeding and at appropriate intervals thereafter.

All heifers which have been vaccinated for six months or more shall be included on the quarantine release test. Those tested on the quarantine release test shall be included in the six month post-quarantine release test.

**ADDITONAL UM&R CHANGES**

1. Mandatory first point testing in B and C states (testing of adult cattle at the first point of concentration for sale, usually the market). A test is required on all post-parturient cows entering quarantined feed lots.
2. Extended quarantine of infected herds to 180 days (6 months post-quarantine retest would still be required).

**RECOMMENDATIONS:**

1. Adjacent herd testing to extend to more than fence contact herds.
2. Immediate compliance with present UM&R and prompt prosecution of violations.
3. Evaluation procedures for C and B States (to be completed quarterly).

1. **Adjacent herd testing**

   \( \frac{\text{adj. herds tested}}{\text{adj. herds eligible for test}} \times 100 = \)

2. **Frequency of retests**

   \( \frac{\text{# of vaccinated herds exceeding 180 day retest}}{\text{# of vaccinated herds}} \times 100 = \)

   \( \frac{\text{# of vaccinated herds exceeding 12 month retest}}{\text{# of non-vaccinated herds}} \times 100 = \)
3. Length of quarantine
   (a) (1) Gross
      \[
      \frac{\text{Accumulative infected herds in 12 months}}{\text{Quarantined herds at a given date}} = \text{ratio}
      \]
   (2) Adjusted rate — subtract newly infected herd totals from each of the above categories

   (b) \# of herds under quarantine for longer than 2 years.
      (These procedures quantify the length of time herds are staying under quarantine in a State. The more quickly herds are cleaned up and released, the higher the ratio will be. Conversely, a State that has herds that remain infected for extended periods of time will have a low figure for this ratio.)

4. Postquarantine release testing
   (a) \# of herds with PQ test \times 100 = \%
      \# of herds eligible for PQ test
   (b) \# of herds found infected on PQ test \times 100 = \%
      \# of herds tested for PQ test

5. Reactor identification rate
   (a) \# of MCI reactors untraceable \times 100 = \%
      \# of MCI reactors disclosed
   (b) \# of MCI reactors disclosed \times 100 = \%
      \# of MCI samples tested

BRUCELLOSIS SCIENTIFIC ADVISORY COMMITTEE
Use of the Buffered Acidified Plate Antigen in Brucellosis Eradication Program

Dr. Joe Rinehart

It is the opinion of the Brucellosis Scientific Advisory Committee that Dr. Rinehart's recommendations for altering the UM&R with regard to the BAPA and RST be adopted only for classifying negative as stated (see (a) insert F. for Chapter 1, UM&R, Part I, C. Negative, Section 1 (b) insert f. under Section 2, (c) deletion under section 2 and (d) deletion Chapter 1, Part II, I. classification section 8.)

However, with regard to the Veterinary Services Memorandum No. 551.19, it is the opinion of the Brucellosis Scientific Advisory Committee that rather than delete the entire paragraph only delete "or herds with presumptive evidence of infection" (suspicious brucellosis ring test marked cattle identification reactor, contact herd, epidemiological traces, etc.).

Furthermore, it is our opinion that the RST be phased out as a presumptive test for classification of negative cattle because of high titer
prozone reactions which cause an excessive number of false negative results.

REPORT OF THE SUBCOMMITTEE
ON BRUCELLOSIS EDUCATION

The subcommittee on Brucellosis Education met at 7:00 a.m. Monday, October 28, 1985.

Reports were given by each member of the committee with respect to his/her educational efforts in his/her state. In addition, each member cited the areas that he felt needed further intensification. Significant discussion centered around the issue of educational efforts and public awareness preceding changes in proposed regulatory actions. Specifically, the committee makes the following recommendations to the full Brucellosis committee:

1. It is recommended that the subcommittee compose an abstract of the minutes of the full committee's actions on issues such as the proposed regulatory changes on heifers. The abstract upon approval of the chairman of the Brucellosis Committee would then be distributed for usage by appropriate media and groups. Members of the Brucellosis Committee would be urged to disseminate and obtain maximum utilization of this abstract of minutes.

2. The committee unanimously recommends that a fact sheet entitled "Purchasing Replacement Heifers" by Dr. Brian Espe of Kerr Foundation be adopted as the most appropriate document to guide producers in avoiding infection.

3. The committee cited the efforts of Dr. Bill Alexander for his innovative work with financial lenders in Oklahoma and called for similar action in other states. Appropriate material to aid in such efforts is underway.

4. The committee felt that information will be a key factor in preventing new infection when the rebuilding phase of the cattle cycle begins. Material developed by APHIS, LCI and Kerr Foundation and efforts to reach ag lenders with such materials will be of critical importance at that time.

5. The committee called for an intensification of efforts by LCI, APHIS, and the Kerr Foundation in developing and obtaining national usage of materials issued in their respective newsletters.

6. The committee cited the helpful work of Dr. George Meyerholz, National Extension Veterinarian, in distributing Kerr Foundation brucellosis material and called for increased efforts by Extension in disseminating information about the disease at national, state and county levels.

7. The committee called for increase and intensified work with 4-H and Vocational Agriculture (FFA).

8. The committee recognized the socio-economic study of the Kerr
Foundation that vividly pointed out the lack of adequate penning and working facilities in Oklahoma and Arkansas.

9. The committee also stressed the importance of whole herd health programs as a way to increase vaccination rates, management practices and to improve the willingness of producers to cooperate with the program.

Mr. Chairman, the sub-committee on Brucellosis Education moves that this report be adopted.

U M & R
Part II
Section R
Page 30

Change from –

Included in the individual herd plan shall be provisions for the herd to be tested within 30 to 120 days following vaccination, and at not . . . .

TO:

Included in the individual herd plan shall be provisions for the herd to be tested within 180 days following vaccination, (however, upon written request by the herd owner the time of the first post vaccination herd test may be extended to coincide with the anniversary date for recertification.) and at not . . . .
A REVIEW OF RESTRICTION ENDONUCLEASE ANALYSIS
AS AN APPLICATION TO THE IDENTIFICATION AND
CLASSIFICATION OF LEPTOSPIRES

A. B. Thiermann, DVM, PhD; R. B. Le Febvre, PhD
Ames, IA

SUMMARY

Restriction endonuclease analysis (REA) was used to classify leptospiral isolates belonging to serogroups Australis, Mini, Pomona, and Sejroe. All isolates selected for this study had been classified by serotyping, and were identified as belonging to serovars bratislava, szwajizak, pomona, balcanica, and hardjo. However, in many cases, the results of REA suggested a different classification.

Two isolates from Oregon serotyped as szwajizak are suggested to be classified as georgia by REA. The REA patterns of North American isolates belonging to balcanica and hardjo were indistinguishable, but different from both reference strains. These isolates have been suggested to be classified into a new subtype (genotype) hardjo-bovis. United Kingdom isolates belonging to serovar hardjo show two different REA patterns; one that matches that of the reference strain, and one that matches hardjo-bovis from North America.

Isolates belonging to serovar pomona from North America and the United Kingdom were examined by REA. Differences were observed between the two origins. All United Kingdom isolates matched reference strain pomona, whereas all North American strains matched reference strain kennewicki. The REA confirmed the identity of bratislava isolates obtained by serotyping.

The REA has proven to be a more discriminating method for the classification of leptospires. Since it analyzes the genetic make-up, it eliminates the cross-reaction problems encountered with all serologic typing methods. It is suggested that new isolates should be classified into a serogroup by serologic methods. Final classification should then be conducted by REA.

The classification of the genus Leptospira, at present, is based on serotyping.1 Using the microscopic agglutination test (MAT),2 reactions of the leptospires with specific antisera for reference groups and serovars is measured. The reliability of this method is subject to nutritional and environmental influences on the culture being typed. A system of antigenic factor analysis using specifically absorbed sera was proposed by Kmety3 for the classification of leptospires. This method is complex, and results are difficult to reproduce in different laboratories.

The application of restriction endonuclease analysis (REA) to the classification of leptospires was first proposed by Marshall et al.4 After improve-

No endorsements are herein implied.
ments in the DNA extraction procedure and the resolution of the restricted DNA fragments in the agarose gel in our laboratory, the REA has proven to be a more sensitive and discriminating taxonomic tool than serotyping. Field isolates from the United States, Canada, and the United Kingdom belonging to serogroups Australis, Mini, Pomona, and Sejroe have been examined and classified according to their REA patterns.

**Materials and Methods**

**Leptospiral strains and isolates** — The serovar reference strains belonging to serogroups Australis, Mini, Pomona, and Sejroe were obtained from the Center for Disease Control (CDC) in Atlanta, Georgia, or the Royal Tropical Institute in Amsterdam, The Netherlands (Table 1). The field isolates were serologically typed at the laboratory of origin as belonging to serovars balcanica, bratislava, hardjo, pomona, and szwajizak. The origin and host of the isolates is described in Table 2.

**Preparation of whole-cell DNA** — High molecular weight chromosomal DNA was prepared according to the method previously described. Leptospires were harvested from 150 ml of an exponentially growing culture in EMJH medium. The DNA of some of the field isolates was extracted by the rapid method described by Le Febvre et al. After extraction and dialysis, the concentration of DNA in each sample was determined by spectrophotometry.

**Restriction endonuclease digestion of DNA** — Purified leptospiral DNA (2 μg) was mixed with 4 to 5 units of restriction enzyme in a 20 μl reaction mixture. Restriction enzymes were purchased from Bethesda Laboratories Inc., Gaithersburg, Maryland, and reaction conditions were those recommended by the manufacturer. After the addition of 5 μl of tracking dye (0.1% bromphenol blue, 20% Ficoll type 400 in distilled water), the samples were electrophoresed at 60 volts for 16 hours on a 20 x 25 cm horizontal electrophoresis box (Bethesda Research Laboratories, Model H-4). Gels consisted of 0.7% agarose in Tris borate buffer. The gels were stained in ethidium bromide for 45 minutes, and photographed under shortwave ultraviolet light through a Kodak 23A red filter.

Twelve restriction enzymes (Bam HI, Bgl II, Eco RI, Hind III, Kpn I, Pvu II, Sal I, Sma I, Sst I, Xba I, and Xho) were screened for cleavage of leptospiral DNA. Complete digestions and best resolutions were obtained with Eco RI, Hha I, Bgl II, and Hind III. Therefore, these four enzymes were chosen for digestion of DNA on all strains in the present study.

**RESULTS**

The two Oregon isolates serotyped as szwajizak were examined by REA and compared to the reference strains in serogroup Mini (Fig 1). Digestions with enzymes Eco RI or Hha I indicated that, these isolates had REA patterns identical to that of reference strain of serovar georgia, and not of szwajizak.

All isolates serotyped as hardjo and balcanica from the United States and Canada gave the same REA pattern when cleaved with Eco RI (Fig. 2).
However, their pattern differed from *hardjo* and *balcanica* reference strains. Minor differences were observed in the pattern of some isolates from Florida and one from Nebraska when cleaved with Hha I (Fig 3). However, the REA pattern still does not correlate with that of the reference strains.

It has been proposed to identify this group of isolates as genotype *hardjo-bovis* with subtypes A, B, and C (Fig. 3). Subtype A is the most common REA pattern found among North American *hardjo* and *balcanica* isolates (61 of 87 isolates tested). All isolates from Canada, Iowa, Colorado, and Illinois belong to subtype A. Among the Florida isolates, 35 of 60 belong to subtype A (*4 balcanica* and 31 *hardjo*) and 25 of 60 belong to subtype B (*4 balcanica* and 21 *hardjo*). So far, only one isolate from Nebraska belongs to subtype C.

Isolates from North America serotyped as *pomona* were classified as *kennewicki* by REA, whereas the *pomona* isolates from Northern Ireland had an REA pattern that matched serovar *pomona* reference strain (Fig 5).

Recently, two Iowa isolates were serotyped as *bratislava*. This identification was confirmed by REA (Fig 6).

**Discussion**

All reference strains in serogroup Mini could be differentiated by REA when using enzymes Eco RI and Hha I. Two bovine isolates from Oregon, which had been initially serotyped as *szwajizak*, were classified as *georgia* by REA. This reclassification of the Oregon isolates 222 and 814 is a result of the greater discriminating power of REA. A high degree of cross-reactivity is observed between *szwajizak* and *georgia* by serotyping. These REA results more closely relate to epizootiologic findings, in that serovar *szwajizak* has never been described in North America before or since this occurrence; however, it is a common pathogen of rodents and occasionally of cattle in Israel. Serovar *georgia* is native to the United States, having been isolated from raccoons, opossums, and skunks.

The presence of serovar *bakanica* in Florida has also been questioned on epizootiologic grounds. *Balcanica* is a common pathogen of opossums, and occasionally of cattle, in New Zealand and Australia. It does not establish persistent infection in cattle. However, in Florida, where opossums are abundant, serovar *bakanica* has been described only in cattle. The REA patterns of these *bakanica* isolates do not match that of *balcanica* reference strain; however, they are undistinguishable from the REA patterns of *hardjo* isolates. The North American *hardjo* and *balcanica* isolates, with REA patterns different from both reference strains, have been proposed as a new type (*hardjo-bovis*) with strain 93-U as reference strain. After studying 87 North American *hardjobovis* isolates, they have been subdivided into subtypes A, B, and C when analyzing the REA pattern after cleavage with enzyme Hha I. The significance of these minor differences is not known. It does not correlate with the differences in serotyping. These subtypes are distinguished by one band position difference when cleaved with Hha I.
Although no hardjo isolate from North America was found to have an REA pattern that matches reference strain hardjoprajitno, over half of the hardjo isolates from the United Kingdom studied so far have an REA pattern that matches or is similar to hardjoprajitno (Fig. 4). These strains identified as hardjoprajitno by REA appear to have special nutritional requirements and were isolated when using Ellis E-5 medium and not with the conventional EMJH medium.

Isolates from North America and Northern Ireland belonging to serovar pomona were also examined by REA and compared to reference strains in serogroup Pomona (Fig. 5). When cleaved with enzyme Eco RI, all isolates from North America showed an REA pattern that matches that of serovar kennewicki. However, all isolates from Northern Ireland matched serovar pomona (Fig. 5). The same results were observed when cleaved with enzyme Hha I (data not shown). Although the differences in REA pattern between kennewicki and pomona are subtle, they are consistent and correlate with epizootiologic findings. Serovar pomona, in the United Kingdom, takes permanent residence in wildlife and causes reproductive disorders in horses. It has also been isolated in a few single incidences in swine. Conversely, in North America, serovar kennewicki takes permanent residence in swine, causing reproductive disorders and chronic shedding. It will occasionally infect cattle and wildlife.

Recently, leptospires serotyped as bratislava were first isolated in the United States. They were isolated from the reproductive tract and kidney of sows in Iowa. When these isolates were examined by REA and compared to reference strains in serogroup Australis, they matched the pattern of bratislava, thus confirming the presence of this serovar in this country (Fig. 6).

After examining over 300 leptospiral isolates by REA and comparing them to the reference strains of five different serogroups, we concluded that this new classification method is rapid, discriminating, and more closely relates to epizootiologic findings.

Once an isolate is grown in liquid broth, the DNA can be extracted and a final classification by REA can be obtained within 5 days; thus eliminating time-consuming rabbit hyperimmunizations.

Since this method examines and compares leptospires by its genetic makeup, rather than its surface components, it eliminates the undesirable problem of cross-reactions characteristic of most serologic classification methods. Because REA is not a serologic method, we should incorporate a new epithet “genotype” to replace “serovar” when identifying a distinct REA pattern. A genotype identifies an organism or group of organisms whose REA pattern can be differentiated from that of all other members in its serogroup by two or more restriction enzymes digestions.

Serotyping has been a useful tool in the classification of leptospires, and should continue to be used for the identification of an organism within a serogroup. Final classification should be done by REA. We suggest that differences or similarities in REA pattern be analyzed after two or more
separate enzyme digestions. As observed in U.S. hardjo isolates, some differences were only evident with one enzyme digestion. This could be the result of a single base pair deletion or substitution, and may not relate to any significant phenotypic differences. Nevertheless, any and all REA pattern differences observed should be noted until further knowledge is gained.

We believe that REA is not a perfect method, but it may be used to considerable advantage in conjunction with conventional serologic methods. Possibly, in the future, the development of specific DNA probes could lead to an even more accurate classification.

ACKNOWLEDGEMENTS

The authors thank W. A. Ellis, B. F. Kingscote, R. M. Nervig, C. R. Sulzer, and F. W. White for supplying some of the isolates, A. L. Handsaker, and J. W. Foley for excellent technical assistance.

REFERENCES


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**TABLE 1---List of leptospiral serovar reference strains within each serogroup**

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<tr>
<th>Serogroup</th>
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**Mini**

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### TABLE 1---List of leptospiral serovar reference strains within each serogroup, continued

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Fig 1 — Electrophoretic patterns of chromosomal DNA from reference strains in serogroup Mini and field isolates Oregon 222 and 814 digested with Eco RI (lanes 1 through 8). Reference strains mini (lane 1), beye (lane 2), tabaquite (lane 3), perameles (lane 4), szwajizak (lane 5), georgia (lane 6), 222 (lane 7), and 814 (lane 8). Digestion with Hha I (lanes 9 through 16). Reference strains mini (lane 9), beye (lane 10), tabaquite (lane 11), para-
meles (lane 12) szwajizak (lane 13), georgia (lane 14), 222 (lane 15), and 814 (lane 16).
Fig 2 — Electrophoretic patterns of chromosomal DNA from North American *hardjo* and *balcanica* isolates and corresponding reference strains digested with Eco RI. Reference strain *hardjo* (lane 1); Colorado isolate 93U (lane 2); Iowa isolates 5296 (lane 3), 81 (lane 4); Canada isolates LC81-30-3 (lane 5), LC82-13 (lane 6), LC81-30-1 (lane 7); Florida isolates 45 (lane 8), 49 (lane 9), 107 (lane 10), 523 (lane 11), 74 (lane 12), 131 (lane 13), 24 (lane 14), 397 (lane 15), 250 (lane 16), 367 (lane 17); Nebraska isolate 18171 (lane 18), and reference strain *balcanica* (lane 19).
Fig 3 — Electrophoretic patterns of chromosomal DNA from North American *hardjo* and *balcanica* isolates and corresponding reference strains digested with Hha I. Reference strain *hardjo* (lane 1); Colorado isolate 93U (lane 2); Iowa isolates 5296 (lane 3), 81 (lane 4); Canada isolates LC81-30-3 (lane 5), LC82-13 (lane 6), LC81-30-1 (lane 7); Florida isolates 45 (lane 8), 49 (lane 9), 107 (lane 10), 523 (lane 11), 74 (lane 12), 131 (lane 13), 24 (lane 14), 397 (lane 15), 250 (lane 16), 367 (lane 17); Nebraska isolate 18171 (lane 18), and reference strain *balcanica* (lane 19).
Fig 4 — Electrophoretic patterns of chromosomal DNA from Northern Ireland isolates, reference strain *hardjo*, and Colorado isolate 93U (hardjobovis) digested with Eco RI (lanes 1 through 9). Reference strain *hardjo* (lane 1); Northern Ireland isolates 80-184 (lane 2), 81-358 (lane 3), 83-969 (lane 4), 81-933 (lane 5), 83-893 (lane 6), 83-901 (lane 7), 83-903 (lane 8); Colorado isolate 93U (lane 9). Digestions with Hha I (lanes 10 through 18). Reference strain *hardjo* (lane 10); Northern Ireland isolates 80-184 (lane 11), 81-358 (lane 12), 83-969 (lane 13), 81-933 (lane 14), 83-893 (lane 15), 83-901 (lane 16), 83-903 (lane 17); and Colorado isolate 93U (lane 18).
POMONA

HhaI

Fig 5 — Electrophoretic patterns of chromosomal DNA from serovars in serogroup Pomona reference strains and field isolates from Northern Ireland and North America digested with Hha I. Reference strains proechimys (lane 1), mozdok (lane 2), tropica (lane 3), tsaratsova (lane 4), monjakov (lane 5), kennewicki (lane 6), pomona (lane 7); Northern Ireland isolates 80-1101 (lane 8), 80-1065 (lane 9), 80-1503 (lane 10), 82-1653 (lane 11), 82-698 (lane 12); reference strain kennewicki (lane 13); North American isolates Po-skunk (lane 14), 5289 (lane 15), RM-70 (lane 16), RM-211 (lane 17), 27-U (lane 18), RM-39 (lane 19); and reference strain pomona (lane 20).
Fig 6 — Electrophoretic patterns of chromosomal DNA from serovars in serogroup Australis reference strains and bratislava isolates from Iowa digested with Bgl II. Reference strains australis (lane 1), lora (lane 2), bangkok (lane 4), peruviana (lane 5), pina (lane 6), nicaragua (lane 7), ramusi (lane 8), muenchen (lane 10), jalna (lane 11), bratislava (lane 12); Iowa isolates 26 kid. (lane 13), 30 ut. (lane 14), and 30 kid. (lane 15).
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

Chairman: H. L. Rubin, Kissimmee, FL
Vice-Chairman: J. R. Cole, Tifton, GA

B. O. Blackburn, IA; S. L. Diesch, MN; John Finnell, IL; J. C. Frantz, NE; J. W. Glosser, VA; R. E. Hall, GA; L. E. Hanson, IL; C. M. Hibbs, NM; C. A. Kirkbride, SD; D. A. Miller, IA; R. L. Morter, IN; R. M. Nervig, IA; J. G. Songer, AZ; A. B. Thiermann, IA; D. H. Tripathy, IL; J. M. Williams, MO

The USAHA committee on Leptospirosis met on October 30, 1985 with 20 members and guests in attendance.

Dr. Alex Thiermann reported on the use of restriction endonuclease analysis of field isolates of leptospires. His presentation is included in the proceedings.

Dr. James England, Chairman of the American Association of Veterinary Laboratory Diagnostician (AAVLD) Interpretive Serology Committee, requested assistance and guidance on the standardization of the microscopic agglutination test (MAT) for leptospirosis serology. Problem areas identified were standardization of antigens, subjectivity of reading the test, and determination of final dilutions. It was concluded by the USAHA committee that the majority of the problems were related to the preparation and standardization of antigens and the use of proper controls. This committee further recommended that the procedures described in the monograph on the microscopic agglutination microtiter procedure, which was published in the 1979 USAHA Proceedings, should be followed by all laboratories performing leptospirosis serology.

Dr. Dave Miller of the Diagnostic Section of the National Leptospirosis Reference Center reported that a protocol describing the MAT was being prepared and would be distributed to the various diagnostic laboratories. In addition, the Diagnostic Section will provide voluntary check-test serum samples, reagents, and training when requested.

The Committee was pleased to note that the Diagnostic Section of the Center had complied with the Committee's 1984 recommendation, and has widely disseminated information about the services provided to the diagnostic community.

Dr. Dave Miller updated the Committee on the activities of the Diagnostic section of the Center. These activities included: 1) Serology, 2) Isolation and Serotyping, 3) Antiserum Production, 4) Reagents Dispensed, 5) Epidemiological Consultation, 6) Training, and 7) Developmental Projects.

Dr. Alex Thiermann updated the Committee on the activities of the Research Section of the Center. These activities are: 1) Restriction Endonuclease Analysis of Field Isolates, 2) Identification and Preservation of New Isolates, 3) Characterization of Leptospiral Surface Proteins, 4) DNA

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The subcommittee appointed at the 1984 committee meeting to develop a list of research priorities on animal leptospirosis presented its report. This subcommittee was chaired by Dr. J. Glenn Songer.

This subcommittee members and three producer groups were formally polled regarding priorities for research on leptospirosis. The results were ranked according to the number of times they were mentioned by the respondents. They are as follows.

1. Improve methods for isolation and propagation of leptospira, especially the fastidious, host-adapted strains. This will benefit both the diagnostician and the biologics industry.

2. Improve other diagnostic methods, especially as pertains to rapid screening of animals for infection status.

3. Make provision for continuing surveillance by way of slaughter house surveys and other appropriate methods, of the prevalence of leptospirosis in swine, cattle, and horses. In addition, efforts to isolate and type leptospira from humans should be increased. A virtual unknown is the economic impact of leptospirosis and information from this research could yield a reasonable estimate of dollar losses.

4. Improve immunogens, particularly as subunit vaccines, investigating possibilities for cross-protection among serogroups by isolated antigens. Allied with this should be efforts to develop and seek approval for in vitro assays of bacterin potency.

5. Continue to improve methods to typing isolates of leptospira, e.g., the restriction endonuclease assays being pursued at the National Animal Disease Center.

6. Investigate pathogenesis of leptospirosis in at least two areas:
   a. Involvement of toxic factors in the pathogenesis of acute leptospirosis.
   b. Microecology of host-adapted strains of leptospira (e.g., serovar hardjo in cattle and serovar bratislava in swine) in the genital tract. This should include studies of attachment and multiplication, as well as the effect of the local and systemic immune responses on these processes.

It is the feeling of the Committee that funding for research on leptospirosis must be increased. Both ARS and APHIS should provide support for increased intramural and extramural research. To this end, a resolution has been prepared for consideration by the membership of USAHA.

The Committee was appraised of reports of the changes in leptospirosis epidemiology. Today, the human groups most at risk are the farm workers and veterinarians who have direct contact with cattle and swine. It was
reported that between 1983–85 that 50% of all human cases of leptospirosis reported in Great Britain were caused by *L. hardjo*, which is responsible for decreased milk production and abortion in cattle. The Committee again wishes to point out the need for federal and state regulatory officials to inform their field staffs of the potential dangers of leptospiral infections which may be contracted when working with cattle and swine.
USE OF THE INTRAMAMMARY DEVICE 
IN THE CONTROL OF MASTITIS

Max J. Paape 
Milk Secretion and Mastitis Laboratory, Agricultural Research 
Service, USDA, BARC-East, Beltsville, Maryland 20705

INTRODUCTION

The ability of polymorphonuclear neutrophil leukocytes (PMNL) to phagocytose foreign particles is a natural defense against pathogens invading the mammary gland. PMNL enter the mammary gland from the blood via diapedesis through the secretory epithelium. The ability of PMNL to phagocytose and kill invading pathogens is compromised by a deficiency of specific antibodies in milk and loss of pseudopods and lysosomal granules as a result of ingestion of fat globules and casein. As a result, large numbers of PMNL (900,000/ml) are required for phagocytic defense of the mammary gland. Localized recruitment of PMNL into the mammary gland was accomplished by inserting an abraded polyethylene intramammary device into the gland cistern. The resulting leukocytosis was shown to be protective against establishment of infection by mastitis pathogens in experimental challenge studies and in commercial dairy herds.

LEUKOCYTIC DEFENSE OF THE BOVINE MAMMARY GLAND

Polymorphonuclear neutrophil leukocytes (PMNL) and macrophages comprise 80–90% of the cells in milk from non-infected mammary glands. In early studies, Schalm et al. reported that a concentration of 500,000 leukocytes/ml of foremilk protected against experimental infection by Aerobacter aerogenes.

Irritation of the lactating mammary gland will dramatically increase the number and percentage of PMNL in milk. However, a time delay occurs between the initiation of irritation in the mammary gland and the appearance of PMNL in milk. This delay was 24 hours after the injection of bacteria into the mammary gland cistern of cows. Further, viable bacteria were recovered until the leukocyte count exceeded several million/ml milk or until the leukocyte:bacteria ratio was very high. Therefore, though milk leukocytes were essential for defense against microbial invasion, because of the low competence of those milk leukocytes, large numbers were required for effective defense.

This observation initiated a number of studies into the phagocytic and bactericidal properties of milk leukocytes. Results from these studies show that leukocytes isolated from milk had lower phagocytic and bactericidal properties than leukocytes isolated from blood had. The reduced phagocytic and bactericidal properties have been attributed to: reduced glycogen stores in milk leukocytes compared to blood leukocytes; deficiency of specific antibodies in milk; and loss of pseudopods and lysosomal granules as a result of phagocytosis of fat and casein.

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ENHANCEMENT OF LEUKOCYTIC DEFENSE

Smooth Intramammary Device — In an effort to increase the leukocytic defense of the udder Paape et al., used a mechanical means to produce a sterile inflammation and thus induce a leukocytosis by inserting a smooth polyethylene device (IMD) into the mammary gland cistern. The IMD measured 2.5 mm in diameter, 115 mm in length and formed a loop of 25 mm in diameter in situ. The principle involved was to induce a leukocytosis in that fraction of milk (foremilk or strippings) closest to a pathogen’s point of entry. To avoid any change in the composition of the milk from the entire mammary gland, any cellular or humoral response to the IMD must be confined to the area of the mammary gland cistern. Milk in the cistern is normally discarded by the dairyman before milking, during the mastitis screening process.

Field evaluation — The effectiveness of the smooth IMD against naturally occurring infections under field conditions was evaluated. No difference was observed in the new infection rate between IMD and control groups. The inability of the smooth IMD to reduce new infections was due to a failure of the IMD to increase milk somatic cell counts (MSCC) in stripping milk to levels needed for protection, considered to be 900,000/ml of milk or greater.

Microscopic examination of smooth IMD — Examination by scanning electron microscope (SEM) of smooth IMD removed from mammary quarters of cows revealed adhered macrophages and PMNL over areas of the IMD that had become abraded by the cannula during insertion of the IMD. It is recognized that the macrophage plays a key role in recruitment of PMNL and adherence of macrophages to IMD appeared to be a prerequisite for promoting infiltration of PMNL into the gland and teat cisterns.

Abraded IMD — The smooth IMD was modified by abrading the entire surface of the device. The modification had the desired effect of increasing MSCC in strippings to 900,000/ml (Table 1). Although MSCC in bucket milk was higher in IMD quarters when compared to control quarters, the magnitude of the increase was small and probably of no immediate concern.

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<tr>
<th>FRACTION</th>
<th>ABRADED (CELLS X 10^3/ML)</th>
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<td>STRIPPINGS</td>
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Experimental challenge studies — The effectiveness of AIMD in prevention of establishment of infection was tested by intramammary injection of mastitis pathogens. Mammary quarters containing either
abraded IMD, smooth IMD or no IMD were challenged with either *Streptococcus uberis*, *Escherichia coli* or *Staphylococcus aureus*. Results are shown in Table 2. Among quarters containing abraded IMD 59 to 68% never became infected. Eighty to 100% of the control quarters or quarters containing smooth IMD became infected. The results from these studies indicate that abrading the surface of the IMD increased MSCC in stripping milk to concentrations that provided good protection.

### TABLE 2.

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<td>(%)</td>
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<tr>
<td><em>S. aureus</em></td>
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</table>

**Field studies** — The abraded IMD is being tested in commercial dairy herds in Israel. This field trial is supported by BARD (United States-Israel Binational Agricultural Research and Development Fund) and in September 1986 will be entering its fourth and final year.

Milk production, MSCC, incidence of clinical mastitis and new intramammary infection rate will be determined on 3,000 cows with abraded IMD in all 4 quarters and on 3,000 control cows. To date thirteen dairy herds totaling 3,660 Friesian dairy cows are on the study. Three of the herds (809 cows) were considered to be mastitis problem herds and were experiencing outbreaks of clinical mastitis when placed on the study. Abraded intramammary devices were inserted into all 4 quarters of cows with odd numbered ear tags. Within a herd, all of the AIMD were inserted on the same day regardless of stage of lactation or dry period. Cows with even numbered ear tags served as controls. Cows were observed at each milking by either the herdsman or milker for signs of clinical mastitis. Clinical cases with systemic involvement were diagnosed by the veterinarian. Total cow months of observation were 31,340.

There were 164 reported cases of clinical mastitis among AIMD cows and 366 cases among controls. Of these clinical cases, 41 of the AIMD cows and 197 of the control cows had systemic involvement that required treatment. Among the 3 problem herds, there were 9 clinical cases for AIMD cows and 43 for control cows. Seventy percent of the isolates from clinical cases with systemic involvement were coliforms, whereas 69% of the isolates from cases without systemic involvement were *Staphylococcus aureus*.

New intramammary infections caused by non-*agalactiae* streptococci and *Corynebacterium bovis* were significantly less in IMD cows.
ever, infections by coagulase negative staphylococci were higher in IMD cows (Table 3).

**TABLE 3.**

**NEW SUBCLINICAL INTRAMAMMARY INFECTIONS (IMI)**

<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>IMD cows</th>
<th>Control cows</th>
<th>(P) level</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>2.7</td>
<td>2.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Coagulase negative staph</td>
<td>3.6</td>
<td>2.9</td>
<td>.06</td>
</tr>
<tr>
<td>Non-agalactiae streptococci</td>
<td>1.5</td>
<td>2.1</td>
<td>.04</td>
</tr>
<tr>
<td><em>C. bovis</em></td>
<td>.9</td>
<td>1.4</td>
<td>.05</td>
</tr>
<tr>
<td>Others</td>
<td>1.7</td>
<td>1.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total</td>
<td>10.4</td>
<td>10.6</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Monthly milk weights for the first 135 cows in 5 herds completing lactation were recently analyzed. Herds were free of *Streptococcus agalactiae*; infection frequencies for other streptococci and *Staphylococcus aureus* ranged from 5–12% and 8–26%, respectively. Cows were from first to eighth parity; calving dates were from August 1982 to September 1983. A least-squares analysis of variance of cows' mean test day yield for the lactation was conducted. The model included effects for herd, year-season, parity and presence/absence of AIMD. Least-squares mean for AIMD cows was 32.6 kg (\(N = 65\)) compared to 31.0 kg for controls (\(N = 70\)) (\(P < .05\)). Thus, for a 305-day lactation, AIMD cows would produce an estimated 488 kg more milk than controls.

The AIMD appeared to be well tolerated and safe in non-infected quarters of lactating cows. A low yet transient incidence of clots and flakes in foremilk and strippings were observed after abraded IMD were fitted into subclinically infected quarters of lactating cows. Clinical examination of the udders of cows with AIMD at drying off did not reveal any abnormalities. Slightly bloody secretions were found in samples removed 2–8 weeks after drying off in 1.5% of the IMD quarters and in 1.4% of control quarters.

**Unanswered Questions** — While results from experimental and field studies indicate that an IMD placed into the mammary gland cistern of cows will stimulate intramammary defense mechanisms to protective levels, several concerns have surfaced on use of the IMD in dairy cows. In experimental studies at Beltsville blood was observed microscopically in milk from quarters with IMD but not in milk from control quarters (Table 4). Causes for the bleeding and the long term effects of bleeding on udder health and milk production are not known. While the presence of blood microscopically may not be of concern, causes for the appearance of blood and possible long term effect on udder health and milk composition must
be addressed. There is the possibility that modification of the IMD could reduce or eliminate blood in milk from IMD quarters.

**TABLE 4.**

<table>
<thead>
<tr>
<th>QUARTERS</th>
<th>RBC X 10^6/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.24</td>
</tr>
<tr>
<td>AIMD</td>
<td>29.32</td>
</tr>
<tr>
<td>Significance</td>
<td>P &lt; .05</td>
</tr>
</tbody>
</table>

An unexpected observation in the Israeli study was the decline in stripping MSCC in AIMD quarters with advancing lactation. Toward the end of lactation, stripping MSCC were similar in AIMD and control quarters. Thus, any protective effect afforded by the elevated stripping MSCC in AIMD quarters diminished with advancing lactation and was lost in subsequent lactations. Also, when AIMD were inserted at drying off, in the subsequent lactation the stripping MSCC in AIMD quarters was similar to control quarters. Thus, it would appear that the ability of AIMD to generate an elevated stripping MSCC diminished with advancing lactation and during the dry period. The diminished MSCC to the AIMD with time may be related to coating of the AIMD with an amorphous like material which in turn covers the abrasions on the polyethylene surface thus producing a smooth IMD. Examination of AIMD from mammary quarters by SEM suggests initial adherence of leukocytes to the surface of the AIMD, followed by the rapid formation of an amorphous coating. Eventually, the entire surface of the AIMD becomes coated with a smooth layer of material resulting in a diminished MSCC response. The prevention of plaque formation appears critical to maintenance of the MSCC response. Preliminary studies at Beltsville indicate that coating AIMD with sulfur hexafluoride will prevent formation of amorphous material on the polyethylene surface.

Normally, PMNL leave the body storage pools randomly, but preferential emigration occurs at the site of the IMD in response to locally produced chemotactic substances. The chemical mediators released either from the IMD, adhered leukocytes, or surrounding tissue have not been identified. In addition to the localized leukocytosis, the IMD also potentiated transport of IgG2 and IgM, the two immunoglobulins required by PMNL for phagocytosis. Thus, the possibility of providing specific antibodies required for phagocytosis through immunization offers a potentially attractive approach of further increasing efficiency of the phagocytic defense of the bovine mammary gland. The elucidation of specific mechanisms involved in recruitment of PMNL and in enhancing phagocytosis would further increase efficiency of the phagocytic defense of the bovine mammary gland by the IMD.

Elevated milk somatic cell counts causes marked changes to occur in the levels of nearly all major and minor constituents of milk. Somatic cell
counts are routinely collected to ensure that commercial milk supplies are in compliance with the Federal standards for Grade A raw milk. Furthermore, purchasers of commercial milk (cheese plants, co-ops, etc) will commonly pay a premium to those farmers that can market milk with a low MSCC.

Because of the widespread concern in the dairy industry with mastitis and MSCC, the development of an IMD that may be marketed on the basis that it will elevate MSCC (even if limited to foremilk and stripping milk) should be carried out in conjunction with an evaluation of the quality of the milk. Such a study of milk quality would need to evaluate and relate compositional data (milkfat, total protein, casein, whey protein, lactose and total solids) to various measures of milk “quality,” including conductivity, MSCC, microbial populations, pH, proteolysis lipolysis (acid degree value), and functionality (milk coagulation testing).

The localized recruitment of PMN leukocytes by the IMD varies among cows. In the Israeli study, 49% of the cows studied had stripping MSCC below 200,000/ml, 24% were between 201,000 and 500,000/ml, and 27% were over 500,000/ml. In studies at Beltsville, percentages were 33, 11, and 56%, respectively. The causes of the variation among cows in response to the IMD are not known.

Mammary quarters containing IMD appear to be more susceptible to infection by coagulase negative staphylococci than control quarters. Also, weigh jar MSCC from IMD cows infected with coagulase negative staphylococci were reported to be higher than control cows that were infected with coagulase negative staphylococci. Reasons for the increased susceptibility of IMD quarters to coagulate negative staphylococci and for the intensified MSCC are not known.

Cooperative studies between the USDA, Beltsville and the Department of Animal Sciences, University of Maryland are planned to develop an IMD that will provide a minimum stripping MSCC of at least 900,000/ml in 85% of the cows tested throughout lactation and the dry period. The new IMD must be well tolerated by the mammary gland such that the microscopic appearance of red blood cells is eliminated or kept at a minimal level (< 200 RBC/ml of stripping milk). The new IMD should cause no reduction in milk quality. Various modifications to the present IMD will be made. These modifications will include addition of additives to the polyethylene, chemical treatment of the IMD surface and changing the shape of the IMD.

SUMMARY

The concentration of milk somatic cells in stripping milk needed to prevent establishment of intramammary infection (IMI) appears to be 900,000/ml or greater. The original smooth IMD was not effective in preventing new IMI, presumably because of its inability to elevate MSCC in stripping milk to levels needed for protection. Abrading the surface of the IMD increased milk somatic cells in stripping milk to concentrations that provided good protection against establishment of infection after challenge with Strep. uberis or E. coli.
The abraded IMD (AIMD) is currently being field tested in Israel. To date, 3,660 dairy cows have been put on the study, half of which have an AIMD in all four quarters. There were 164 reported cases of clinical mastitis among AIMD cows and 366 cases among controls. Analysis of milk production for the first 135 cows completing lactation indicated that IMD cows produced significantly more milk than control cows. These studies indicate that an intramammary defense mechanism can be successfully enhanced and developed into a practical deterrent against mastitis.

REFERENCES


REPORT OF THE COMMITTEE ON MASTITIS

Chairman: C. A. Jordan, Morgan Center, VT
Vice Chairman: Thomas J. Fuhrmann, Tempe, AZ

J. B. Adams, VA; Joan M. Arnoldi, WI; Ralph W. Bennett, GA; Allan N. Bringe, WI; R. B. Bushnell, CA; M. L. Crandall, MD; Nance E. East, CA; Charles F. Emerick, WA; Carl Graham, MO; Grancis D. Gregerson, CO; J. Wade Groff, PA; Michele C. Howard, CA; D. E. Jasper, CA; Clinton N. Jewett, AR; Darrel E. Johnson, WI; C. A. Kirkbride, SD; J. S. McDonald, WA; T. G. Murnane, TX; John E. Post, CT; Duane N. Rice, NE; Richard Sechrist, OH; F. E. Sterner, CO; G. H. Swenson, MI; L. A. Wager, NY; D. U. Walker, VT; Rufus F. Weidner, IL

The meeting was held at 1:30 p.m. in Milwaukee, WI on October 30, 1985.

The October 22, 1984 Committee report and the meeting of the committee in Las Vegas, February 17, 1985 were approved as read.

The six goals developed last year were reviewed and the following progress reported:

1. Strengthened our working arrangement with the three other national committees by:
   a) Continued to play an active role in the Joint Mastitis Committee made up of representatives in USAHA, NMC, AVMA and AABP. Attended a meeting of that group while at the National Mastitis Council meeting in February 1985 where we agreed to ask the Dairy Cooperatives to help us disseminate mastitis control information by including envelope stuffers with their milk checks and increase our use of the farm media, emphasized that milk quality programs require close cooperation among various elements of mastitis control and urged the National Mastitis Council to design a model state Mastitis Control program.
   b) The chairman is representing USAHA on the National Mastitis Council Board.
   c) We attended and participated in the February 1985 meeting of the AVMA Mastitis Committee.

2. Continued our study of Mastitis Control programs by having Dr. John Kunkel of the University of Vermont update us on the “Vermont Milk Quality Enhancement program.” He reported that awareness meetings and training sessions were held for veterinarians, dairy cooperative fieldmen, state sanitarians, equipment dealers and county extension agents. Stable hygiene milking machines and milking procedures, therapy, culling and economics of prevention were discussed. Training sessions were held to help cooperatives and Veterinary practitioners develop their own mastitis laboratories. A state survey of bulk tank milk was conducted from August through November 1984 where they
found that in 2,931 herds 47% had *Streptococcus agalactia* infection and 33% *Staphylococcus aureus*. Herd problems were 50% inadequate equipment with 38% having major equipment problems, 65% had major infection and 50% were not teat dipping. Vermont employs one field technician who “trouble shoots” for those herds in violation of the state’s one million somatic cell count limit and is available on request for those dairymen desiring to avoid trouble.

3. In order to determine the progress being made in the development of milk quality programs, John Adams reviewed his paper, An Overview of the Monetary Incentive Quality Programs of Dairy Cooperatives which you just heard during this morning’s session.

4. As part of our study of bacteriological and environmental somatic cell count and mastitis herd profile:

   a) Richard Sechrist, Executive Secretary of National DHIA, reported that as of mid-year 1985, 30,000 herds with 2.5 million cows are participating in DHIA monthly mastitis screening programs. Of all herds and cows in the DHIA system, 47% of the herds and 53% of the cows are enrolled while less than ten years ago only 11% were enrolled. National standards for calibration of somatic cell instruments are followed with samples from two non-DHIA labs providing most reference samples for the DHIA industry. These samples are prepared from raw bulk milk preserved with potassium dichromate. The somatic cell dilutions are prepared in the dichromate-preserved skim milk. These calibration samples are stable at refrigerator temperature for many weeks and are not affected by the usual conditions of shipping.

   b) Dr. John Kunkel of the University of Vermont presented a paper on “Subclinical Mastitis and the Effect of Environmental Organisms” in which he asked us to take a fresh look at many of our preconceived views. He reviewed the effect of subclinical mastitis on milk production efficiency, compared mastitis infections caused by the usual contagious pathogens with those organisms in the environment that may cause mastitis noting that many herds today are free of the usual contagious mastitis pathogens but still have significant infection caused by these environmental organisms. He suggested that more research of this problem is needed.

Dr. Tom Furhmann discussed “Mastitis Control programs for Large Dairies in Arizona. He emphasized that “Mastitis is often a disease of man manifested in animals” with Mastitis Control “correcting mismanagement.” He emphasized that in these large dairies, “clear performance standards are important” with quality incentives very essential. He described the monetary quality incentive program of the United Dairyman Cooperative of Arizona whereby each dairymen can earn three cents a hundred more for satisfying the program criteria. At the start of the program 60% of the herds did not qualify but three months later only 30% did not qualify. The following are the standards that have to be met:
1. SCC of < 300,000
2. SPC of < 30,000
3. Clean sediment test
4. No inhibitors
5. Pesticide residue < .05 ppm
6. Aflatoxin < .5 ppm
7. Cryoscope of 0

Dr. Max Paape of Beltsville reviewed the paper you just heard on the “Use of the Intramammary Device in the Control of Mastitis” and also described the Agricultural Research programs dealing with Mastitis at Beltsville. There are programs dealing with studies of the inheritability of resistance to mastitis, the development of effective vaccines and the study of immunology, studies of the natural defense mechanism of the bovine mammary gland and the teat orifice and the influence of antibodies on phagocytosis.

Dr. Geoffrey Westfall, a veterinary practitioner from Connecticut described and demonstrated the use of a chlorhexidene teat dip packaged in an aerosol can which will be available commercially after the first of the year. His research showed that dirty open teat dip containers, as often now used, can spread mastitis. The aerosol method of application will prevent this type of contamination and has the added benefit of helping to shrink the end of the teat after milking.

Dr. John Post of the University of Connecticut added to his report of last year concerning his research on Goat Mastitis and the part the Caprine Arthritis Encephalitis Virus plays in the Hard Udder Syndrome and resulting mastitis in goats. His preliminary studies indicate that caprine arthritis encephalitis (CAE) virus infection causes many cases of interstitial mastitis in goats resulting in elevated somatic cell counts, diminished milk production, udder indurations and the post-parturient “hard udder — retained milk” syndrome.

In a study of 670 goat milk samples from Connecticut submitted for mastitis diagnosis 25.4% had evidence of mastitis based on inflammatory cell counts of 1.5 million or more cells per ml. Bacterial isolates included Staphylococcus aureus 10.4%, Staphylococcus epidermidis 3.1%, other Streptococci 0.7%, and coliforms 0.3%. The remaining 10.9% of mastitis samples were negative for bacteria and were classified as non-specific mastitis.

Subsequent additional studies of many of the non-specific cases have shown them to be negative for mycoplasma as well as bacteria, but positive for CAE virus antigen. These antigens were detected by immunofluorescent tests on cytospin preparations of milk cells. Such goats were also seropositive for CAE virus antibodies. The presence of this virus and the absence of other pathogens suggest that CAE virus is a cause of many cases of non-bacterial mastitis.

In an additional study of post-parturient goats with firm udders, limited milk yield, and non-specific mastitis, CAE virus was isolated by ex-
plating mammary tissue in tissue culture. These isolates were confirmed by the appearance of syncytial cells, immunofluorescent tests, and electron microscopy. Histologic examination of udder tissue revealed peri-ductal and peri-acinar infiltrates of lymphocytes, macrophages, and plasma cells. In some areas these infiltrates formed valve-like protrusions into ducts. After milking, some mammary gland lobules were still distended with stagnated milk while others were drained. This suggests that inflammatory cell infiltrates block some ducts causing milk retention and udder firmness. In more progressive cases there was extensive inflammatory cell infiltrates that obliterated most of the secretory tissue and there was also proliferation of fibrous tissue. Many of these goats had the typical joint lesions of CAE viral arthritis.

Dr. J. R. Harr of USDA's FSIS committee thanked our committee for its work with Dr. Stephen Oliver in proving that antibiotic milk or colostrum would not cause residues in veal veal and for the chairman serving on the veal calf residue avoidance task force.

The committee agreed to continue to work on the problems referred to above and in addition they desire to encourage the development of new and more effective infusion products and vaccine. It was agreed to meet again during the National Mastitis meeting to be held in Columbus, OH during February, 1986 with one of the principal items on the agenda to be the completed survey of the monetary incentive quality programs to be presented by John Adams and the development of an agenda for the 1986 Annual Meeting of the USAHA.
SUBCLINICAL MASTITIS AND THE EFFECT OF ENVIRONMENTAL ORGANISMS

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Associate Professor
The University of Vermont
Burlington, VT 05405

Introduction

I would like to thank Dr. Jordan and the members of this committee for asking me to present my views on changes within the dairy industry that influence subclinical mastitis and the research program we are involved with to respond to those changes. While much is known about environmental mastitis, it is my purpose to convince you that we must take a fresh look at many of our preconceived views.

Present Situation

Mastitis is the single most costly disease in American animal agriculture. Losses are estimated to be in excess of $180 per cow annually or 2 billion dollars per year nationally. This is equivalent to 11% of the total farm sales of milk. Over 70% of this cost is due to subclinical mastitis through reduced milk production. Estimates are that 35% of our cows are infected in one or more quarters and three out of four will retain the infection 75% of their milking life. Mastitis reduces the manufacturing and nutritional quality of milk and meat. Losses due to clinical mastitis are quite obvious; however, interferences with genetic potential and milk marketing are more subtle. A reduction in total milk yield, caused by damage to epithelial cells reducing milk synthesis, changes in levels of lactose, fat, casein and other components are at the root of these losses.

Mastitis is caused by many species of microorganisms. These can be subdivided into two groups: Those which are contagious such as *Staphylococcus aureus*, *Corynebacterium bovis*, *Streptococcus agalactiae* and the mycoplasmas; and those of environmental origin such as coliforms and esculin positive streptococci. The pattern and type of infection is different; depending on the time exposure occurs, how long it lasts, the ability of the agent to colonize within the gland and reaction of the host. The primary reservoir of contagious pathogens, with the possible exception of the mycoplasmas, is the infected quarter, and spreading occurs during the milking process. Infections with coliforms tend to be of short duration and can be contracted between milking or during the dry period. Approximately 80 to 90% of coliform infections and 50% of the streptococcal infections will result in clinical mastitis, and present therapy does little to alter the course of infection.

Udder Hygiene studies include development of new, novel, and effective teat treatment products that reduce the rate of environmental intramammary infections. Pre- and postmilking teat dipping receive the majority of effort.
Natural Resistance to bovine mastitis involving investigation of chemical factors in the teat canal keratin that inhibit invasion by bacteria. Lipids of the keratin and interactions with mastitis pathogens are the major interest area.

Diagnosis and Control studies include work on more efficient and accurate methods: To identify pathogens that cause mastitis; to measure the relationship of SCC to other tests; and to predict milk composition and yields based on the bacterial isolate responsible.

Summary

We are at a new phase in mastitis control. Bacterial culture, isolation and antibiotic sensitivity testing from individual animals will be of less value than monitoring of bulk tank milk. SCC will continue to be used to assess progress in herds with contagious mastitis pathogens as they become more commonly used as a basis of incentive and penalty payment plans.

Premilking and postmilking use of germicidal agents will continue to be used, however, residues will have to be avoided. Dips will never replace the need for good environmental management, good nutrition and special care of heifers and dry cows.

As our ability to measure and record larger amounts of data increases, we will be in a better position to evaluate the cost/benefit of prevention, treatment and control programs. When evaluating subclinical mastitis, the causative bacteria may be the major factor determining the amount of milk and solids lost, not the SCC value itself.

Research is needed to evaluate new ways to reduce exposure, increase resistance or diminish the leucocytic response within the mammary gland. We have the responsibility to see what improvement is made in each of these areas if we are to derive our livelihood from the industry.

Traditionally bacterial culture and isolation have been important in detecting carrier animals with chronic infections. However, diagnosis through culturing detects contagious agents more effectively because they usually have a longer duration of infection. Detection of environmental agents is difficult because isolation of an agent does not necessarily signify or indicate an infection. In addition, coagulase negative staphylococci and Corynebacterium bovis are present in almost all herds. These so-called secondary pathogens or minor pathogens do elevate somatic cell counts (SCC) to levels indicative of lost production.3

Modern surveillance for subclinical mastitis is based on the use of SCC. Numerous researchers verify that milk yield and composition are severely affected by elevated SCC even at very low levels.4,5 Some data indicate that levels of 100,000 in some cows may already have some economic significance while others indicate SCC levels must exceed 500,000 before there is an economic effect.6 This has led to correlating SCC directly with total milk yield regardless of the causative agent. I believe this approach may be too simplistic in managing our dairy herds. Herds with significant
Streptococcus agalactiae infections which are "blitz" treated show a post-treatment increase in production which is cost effective even though the individually infected cows show little response until the next lactation. General treatment of cows with SCC above 400,000, however, is not cost effective; nor is treatment of cows with Staphylococcus aureus.

Recent progress in mastitis control came about by prevention of the spread of contagious infections during the milking process. Application of post-milking teat dips and the use of dry-cow therapy is practiced on almost all profitable dairies. This has reduced the incidence of contagious mastitis and lowered the SCC. However, in herds where the incidence of clinical mastitis has increased, herd cultures often reveal so-called minor pathogens, or esculin positive streptococci. These organisms and the coliforms appear to be the major cause of elevated SCC in "Streptococcus agalactiae and Staphaureus free" herds.

Monthly clinical mastitis rates often exceed the percent of cows with an elevated SCC. Although the organisms do not seem to have the tissue destructive capacity of the Strept. ag. or Staphaureus, treatment is not effective. Iodine teat dips can change teat end microflora, and promote the presence of staphylococcal species, especially Staphylococcus hyicus. The microenvironment of the teat end needs more study.

Research at University of Vermont

Three areas of research are under study to investigate bacterial and environmental influences on SCC and herd mastitis profiles: 1) Udder Hygiene, 2) Natural Resistance, and 3) Diagnosis and Control.

REFERENCES


11. Hogan, J. S., unpublished data.
RESIDUAL VIRUSES IN ANIMAL PRODUCTS
P. D. McKercher, R. J. Yedloutschnig, J. J. Callis and R. Murphy
Plum Island
Southport, NY

There are a variety of animal products on the international market that originate in countries with animal diseases that do not exist in the importing countries. Modern methods of transportations of such products have severely limited or even eliminated some of the natural barriers that helped reduce the possible importation of such diseases. One such product is "Prosciutto di Parma" (Parma ham). The United States Government banned the importation of Parma hams because of the outbreaks of African swine fever (ASF) and swine vesicular disease (SVD) in Italy between 1973 and 1978. Duplicate experiments; one at the Plum Island Animal Disease Center (PIADC), Agricultural Research Service, United States Department of Agriculture and one at the Instituto Zooprofilattico Brescia, Italy showed that processing and maturing of the Parma hams inactivated SVD in 365 days, the minimal curing period for such products. The following experiments were conducted to determine if foot-and-mouth disease virus (FMDV), African swine fever (ASF) and/or hog cholera virus (HCV) would survive the processes used in the curing and maturation of Parma hams. The experiments were again done in duplicate in the U.S. and in Italy. The authors will, at this time, present only the results obtained in the experiments conducted in the U.S.

Materials and Methods

Viruses: (1) FMDV C1 Brescia isolate, 1964; (2) ASFV/Lisbon-60 BC-4, 6/11/80 PIADC 1 (swine) spleen and blood 2/22/84; and (3) HCV CL virus PIADC challenge virus 1:5 NADL V111, PIADC 2, 3/7/84.

Processing: Seventeen pigs, each weighing between 130 and 150 Kg, were used in each experiment. Five noninfected pigs were processed to provide 10 hams to serve as quality controls for each experiment. Twelve infected pigs for each disease were slaughtered at the peak of infection and processed to produce 24 hams.

Hams from each experiment were identified by numbers and placed in separate atmospheric chambers. The hams for each disease were processed at different time periods to avoid any possibility of cross contamination. Hams from all groups were processed (similarly) to the previous experiment on the survival of SVD in Parma hams.

At time of slaughter, meat samples were collected from all infected animals and assayed to determine that the carcass did contain the respective virus. The titer of the FMDV was determined as plaque forming units in IB-RS-2 cell cultures. Samples of muscle, fat and bone marrow were each assayed for viral infectivity in cultures of IB-RS-2 cells in 75 sq. cm Falcon flasks.

The samples from the ASF experiment were examined by the hemad-
sorption test and expressed as Had$_{50}$/g$^3$. The samples from HCV test were inoculated into PK-15 cell cultures and examined by the fluorescent antibody cell culture technique$^3$. The in vitro tests for ASFV and HCV were each conducted in triplicate on different days. The ASFV assays were each subpassaged three times.

One g samples of muscle, fat and bone marrow were each tested for virus survival at various intervals from the time of slaughtering. Negative tests were confirmed by (host animal) inoculation of swine. When two negative host animal tests were obtained at two consecutive time intervals, the respective virus was considered to be no longer viable. The sera of the inoculated test animals were examined for antibody content and the immunity of the animals challenged with a homologous virus.

Results

(1) In the FMD experiment, virus was recovered from the muscle, fat and bone marrow from samples taken at time of slaughter; however, FMDV could not be recovered at 72 hours from the muscle. In the 30 day sample FMDV was recovered in the bone marrow only.

All of the 90 and 120 day samples were negative for FMDV in tissue culture and also were negative by animal inoculation. Antibody to FMDV was not detected in the sera of the inoculated swine and they were susceptible to challenge with the respective FMDV. (2) The ASFV proved to be a rather resistant virus not easily inactivated by the curing process. The virus was recovered from all samples at 0, 30, and 90 days but only from the muscle and fat samples at 120 days. At 180 days ASFV was recovered from the muscle and fat of 2 of 3 infected hams. At 300 days no virus could be recovered in vitro. However, the pooled samples infected inoculated pigs. The 400 and 430 day samples were negative in vitro and in vivo. Pigs inoculated with pools of the 400 and 430 day samples did not produce antibodies and were susceptible to infection on challenge of immunity. (3) HCV was recovered from all samples at time of slaughter and at 0 days in vitro and 2 of 3 swine in fat and bone marrow samples at 30 days. The samples taken at 90 days were negative in vitro but the pooled samples infected pigs on inoculation. The samples taken at 180 days were negative in vitro and positive when inoculated into pigs. The samples from infected hams at 300 and 365 days proved negative both in vitro and in vivo (Table 1).

Discussion

In all three experiments, as in the initial experiment on the survival of swine vesicular disease, the infected animals were slaughtered when the carcasses contained the greatest amount of virus! Thus, these experiments are examples of a "worst case situation" and in general would contain more virus than would probably occur under more normal circumstances. Investigations of outbreaks have incriminated the feeding of garbage containing meat scraps contaminated with such agents as a major source of infection. Studies with products such as dry salami or pepperoni sausages
produced from pigs infected with SVDV demonstrated that pigs could become infected when fed varying amounts of such products. However, when all of the agents were inactivated or could not be recovered from the processed hams after 300 to 400 days of processing and curing it is very unlikely that such products, whose minimal curing period is 365 days, would be a source of disease transmission. Although the risk of infecting livestock from human food products that could contain residual viruses is small one must be aware that there is a risk, however slight.

REFERENCES


Table 1. In Vitro Testing of Samples

<table>
<thead>
<tr>
<th>Time of Slaughter</th>
<th>SVDV</th>
<th>FMDV</th>
<th>ASFV</th>
<th>HCV</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
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<td>-</td>
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<tr>
<td>90</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>120</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>180</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>-</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>430</td>
<td>-</td>
<td>*</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

+ = Virus recovered.

- = Virus not recovered.

ND = Not tested.

* = Confirmed by animal inoculation.

0–Day = 72 hours post slaughter.
During fiscal year (FY) 1985 (October 1, 1984, through September 30, 1985), there were 480 diagnostic investigations for possible foreign animal diseases in the 50 states and Puerto Rico. Of these, 440 cases were because of suspected vesicular conditions in cattle, horses, sheep, goats, and swine. Four hundred and thirteen of the investigations were made in the last quarter as a result of the recurrence of vesicular stomatitis (VS) type New Jersey in New Mexico, Arizona, and Colorado. The last significant outbreak in the United States took place in 1982–83 and was also the New Jersey type.

Vesicular stomatitis for the year started in December with one horse at Marfa, Texas, that was diagnosed positive for VS type New Jersey based on an increased titer and clinical signs compatible with VS.

The outbreak in three of the southwestern states was first observed in New Mexico, with clinical signs suggestive of VS in horses and cattle during the middle of May. This was confirmed on June 10, 1985, by virus isolation at the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island, New York. The majority of the 64 cases in New Mexico were located in the Albuquerque area. VS in Arizona was first observed in mid-June near Ft. Huachuca, with serological confirmation completed the last of June. Isolation of VS virus from horses located at Pinetop, Arizona, was made on June 30. The first outbreak in Colorado was near Pueblo and was confirmed on July 17 by virus isolation. Colorado had 147 positive cases which occurred on both the eastern and western slopes. Most of the investigations were in the Pueblo area.

All states of the continental United States west of the Mississippi made investigations for possible vesicular conditions except Missouri and Nevada. Investigations were made in 10 states in the eastern United States.

Two field studies were undertaken as a result of the VS outbreak. One was to establish baselines for livestock in Colorado and New Mexico from serums available from laboratory, salebarn, and slaughter sources. The second study was conducted in conjunction with the Centers for Disease Control (CDC) and the Agricultural Research Service (ARS). This study included: examination of insects for virus, livestock titers before and after an outbreak on positive premises, an ELISA test system for on-farm diagnosis, and a fingerprint comparison to other outbreaks. Results of the studies are incomplete.

Veterinary Services in order to assist in the control of the disease supplied updated information of the outbreak to the cooperators. Thirty-day state quarantines were recommended on all premises with positive animals. Fairs and shows in the affected areas were encouraged to control insects with pesticides and observe biosecurity measures if they could not be canceled.
1985 Vesicular Stomatitis Type New Jersey Outbreak in Three Western States

<table>
<thead>
<tr>
<th>State</th>
<th>Investigation</th>
<th>Positive Virus Isolation</th>
<th>Positive Serology</th>
<th>Total</th>
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<tbody>
<tr>
<td>Colorado</td>
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<td></td>
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</tr>
<tr>
<td>104 bovine</td>
<td>32</td>
<td>23</td>
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<td>4 ovine</td>
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<td>2 caprine</td>
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<td>157 equine</td>
<td>20</td>
<td>71</td>
<td>91</td>
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<tr>
<td>Arizona</td>
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<td></td>
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<tr>
<td>2 bovine</td>
<td>1</td>
<td>0</td>
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<td>1 ovine</td>
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<tr>
<td>4 equine</td>
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<td>New Mexico</td>
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<tr>
<td>38 bovine</td>
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<tr>
<td>51 equine</td>
<td>3</td>
<td>33</td>
<td>36</td>
<td>36</td>
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</table>

Of interest was the detection of cases of epizootic hemorrhagic disease (EHD) in cattle when investigating suspected vesicular conditions. Two were in Tennessee and one in Colorado. Since October 1st, the number has increased to twelve in Tennessee.

Final activity for eradication of H5N2 avian influenza in poultry that started in November 1983 was completed April 2, 1985. At least year's meeting, we reported that the area quarantines had been released in Virginia on September 14 and Pennsylvania on October 4, 1984, and that there remained a few Pennsylvania premises under quarantine for cleaning and disinfection completion. A 6-month surveillance program for each area after quarantine was conducted and ended March 15, 1985, in Virginia and April 2, 1985, in Pennsylvania. During the surveillance period, two additional seropositive flocks were detected and depopulated in Pennsylvania. There were no seropositive cases in Virginia poultry nor any virus detection of H5N2 virus in either state.

An additional detection of H5N2 avian influenza in poultry occurred in December 1984. Virus was isolated from live slaughter chickens offered for sale on the streets of Washington, D.C. The chickens and other poultry were seized from two hucksters that were selling without licenses. The virus was not pathogenic to poultry at the National Veterinary Services Laboratories (NVSL), Ames, Iowa, and Dr. R. G. Webster, St. Judes Christian's Research Hospital, reported the virus had similarities to the Pennsylvania virus observed in April 1983. The dealer's premises and the two premises that received the seized poultry were depopulated. Two hundred and fifty backyard flocks in five states were investigated as a result of connections to the dealer with negative results.

The tick eradication program for *Boophilus microplus* and *Amblyomma*
variegatum in Puerto Rico has been making substantial gains. Only three of the latter tick were collected on cattle this year. Tick problems were complicated by the diagnosis of babesiosis in April 1985.

A practitioner collected specimens from a dairy herd on April 1. Babesia bovis-like bodies were observed at NVSL. Two investigators were sent from the laboratory to Puerto Rico. They prepared smears that revealed what appeared to be B. bovis. Two susceptible adult bovine steers at NVSL were used for inoculation studies. On April 29, 1985, laboratory findings were reported as compatible with a diagnosis of bovine piroplasmosis. B. bigemina is commonly detected on blood smears of infected cattle.

On October 1, 258 premises having approximately 18,684 head of cattle are under quarantine for babesiosis in Puerto Rico. Forty infected herds were identified — 16 by slide, 20 with high serological titers, and 4 by close relationship to known infection. One hundred and forty-five herds have positive complement-fixation test results, and these herds are receiving further evaluation.

Spraying on premises with babesiosis infected livestock is being done at 14-day intervals. Resources have been made available to increase spraying in addition to the present tick eradication program to reduce livestock losses. The elimination of babesiosis is dependent upon the elimination of the Boophilus tick from Puerto Rico.

While still in quarantine, a stallion imported from West Germany was found to be infected with contagious equine metritis (CEM) in May 1985 when two test mares were found to be infected. A great deal of treatment effort was necessary to rid him of the disease. The fractious nature of the animal and a localized secondary infection of the prepuce complicated the treatment. Final release of the quarantine was accomplished this month.

Pet birds again this year were found to be infected with velogenic viscerotropic Newcastle disease (VVND). In October 1984, a pet bird shipped from California was found to be infected when examined in Hawaii. Surveillance tracing failed to detect further infection.

Pet birds were found to be infected with VVND on three Florida premises during January 17 through February 26, 1985. Two of the positive premises were commercial wholesale facilities; the third was a private aviary. Tracing of sales from the positive premises disclosed an additional case in Puerto Rico. The tracing of birds from the Puerto Rican premises and from the wholesale dealer in Florida to 25 additional states failed to detect additional cases.

In May, a newly purchased pet bird in a private home in North Carolina was found to be infected with VVND. The infection was also found at the bird's source, a Missouri dealer. The tracing from the dealer failed to detect additional cases.

In February 1985, a causative agent of nematodiasis, Nematodirus battus, was identified from several sheep in the Willamette river valley, west Oregon. This parasite is the most recent addition to the serious
diseases of animals, as one of the economically important foreign animal
diseases and parasites.

Two foreign animal disease diagnostician courses were conducted dur-
ing this fiscal year. Therefore, with the addition of 30 newly trained
diagnosticians, we now have available in the United States a total of 260
veterinarians trained for field investigations of foreign animal diseases.
Four of the 30 are state regulatory veterinarians.

The four Regional Emergency Animal Disease Eradication Organiza-
tions (READEO's) are fully staffed and maintained to respond rapidly to
outbreaks of emergency diseases.

The Technical Support Staff of Emergency Programs has continued the
cooperative agreements with the Southeastern Cooperative Wildlife Dis-
ease Study in Athens, Georgia, and the University of Wisconsin. These
projects are concerned with the role of wildlife in any outbreaks of foreign
animal diseases and for reference in the identification of VVND.

The Technical Support Staff has completed the printing of a new disease
guide, the Avian Influenza Eradication Guide. The Hog Cholera and
Heartwater Eradication Guides have been sent to the printers and will be
available soon.

The Data Bank now has about 54,000 articles on foreign animal diseases
covering 41 diseases and entomological items.
REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

Chairman: G. S. Trevino, Laredo, TX
Vice-Chairman: John L. Hyde, Beltsville, MD

W. W. Buisch, MD; J. J. Callis, NY; C. A. Carson, MO; Robert Combs, NV; A. H. Dardiri, NY; J. B. Finley, Jr., TX; C. M. Grocock, Africa; A. E. Hall, MD; J. A. House, NY; E. W. Jenney, IA; F. M. Jones, FL, D. D. King, MD; K. L. Kuttler, ID; Linda L. Logan, NY; S. McConnell, TX; H. A. McDaniel, MD; P. D. McKercher, NY; N. L. Meyer, VA; James I. Moulthrop, MD; T. G. Murnane, TX; E. I. Pilchard, MD; G. Poppensiek, NY; I. Ross Reid, Canada; S. L. Reynolds, TX; Donald H. Schlafer, NY; E. C. Sharman, MD; A. W. Smith, OR; Peter H. Timm, CA; T. M. Wilson, PA; S. T. Wilson, Jr., DC; John H. Wyss, FL; R. J. Yedloutschnig, NY.

The Foreign Animal Diseases Committee met on Tuesday afternoon, October 29, in the Parliament Room and on Wednesday afternoon, October 30, in the Chagall Room of the Marc Plaza Hotel in Milwaukee, Wisconsin. A standing-room only congregation of members and guests attended both meetings. Space limitations resulted in accommodation for 43 persons on Tuesday and 40 on Wednesday.

A total of 13 papers were presented before the Committee. Names of the discussants are given following the title of their report. Those reports presented at the general session are indicated by an asterisk (*).

<table>
<thead>
<tr>
<th>Date</th>
<th>Title</th>
<th>Discussant</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 29</td>
<td>Emergency Programs Progress Report</td>
<td>Dr. A. Hall*</td>
</tr>
<tr>
<td></td>
<td>Malignant Catarrhal Fever Research</td>
<td>Dr. W. P. Heuschele*</td>
</tr>
<tr>
<td></td>
<td>Residual Viruses in Animal Products</td>
<td>Dr. P. D. McKercher*</td>
</tr>
<tr>
<td></td>
<td>An Ovine Helminth Hitherto Unreported in the U.S.</td>
<td>Dr. G. Zimmerman</td>
</tr>
<tr>
<td></td>
<td>FMD Prevention Activities in Panama and Darien Gap Area</td>
<td>Dr. F. M. Jones</td>
</tr>
<tr>
<td></td>
<td>Global Aspects of Animal Disease</td>
<td>Dr. W. W. Buisch</td>
</tr>
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<td>October 30</td>
<td>Exotic Caliciviruses in the U.S. Owner Survey of Hog Cholera Problems in Honduras</td>
<td>Dr. A. W. Smith</td>
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<td>Bovine Babesiosis Research in U.S. Improved Methods for Heartwater Disease Diagnosis</td>
<td>Dr. E. Hunt McCauley</td>
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<tr>
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<td>United Nations Associate Expert Program</td>
<td>Dr. K. K. Kuttler</td>
</tr>
<tr>
<td></td>
<td>Report on Vesicular Disease Research</td>
<td>Dr. L. L. Logan*</td>
</tr>
<tr>
<td></td>
<td>Epidemiologic Aspects of Vesicular Stomatitis in Mexico</td>
<td>Dr. J. L. Hyde</td>
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<td></td>
<td></td>
<td>Dr. P. D. McKercher</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dr. K. R. Preston</td>
</tr>
</tbody>
</table>

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Global Status of Animal Diseases Exotic to the United States

Foot-and-Mouth Disease (FMD)

In Europe, outbreaks of FMD in Italy created the potential for spread into other countries. At one point, there were over 100 cases per month. The outbreak, identified as Type A5, started in Northern Italy in November, 1984 and spread over the entire length of the country. As recently as August, 1985, there were 12 cases reported.

Fewer cases were reported in Africa. Type A was identified in Ethiopia and Cameroon, Type O in Malawi and Tanzania, and SAT3, in Zimbabwe. In Asia, the incidence of Type Asia1, appears to be on the increase. This type was found in Bahrain, Thailand, Malaysia, Pakistan and Israel. Turkey and Oman reported Type O. Type A22 was also reported in Turkey. In South America, the most prevalent type reported was O, Followed by A and C. Argentina and Brazil lead with the number of cases reported. Chile is again being evaluated by the United States Department of Agriculture for consideration as being FMD free.

African Swine Fever (ASF)

The disease is reported regularly from Spain and Sardinia; however African Swine Fever appeared in Belgium for the first time in early 1985. It was fairly well established that it arrived there in head cheese from Spain. Eradication efforts appear to have been successful and no new cases have been reported in Belgium since May, 1985. The disease does not exist on the Italian mainland, in France, or on the island of Malta where outbreaks have occurred from time to time. Portugal is still considered infected.

In Africa, the disease remains endemic. However, in 1985, there have been no reports of massive outbreaks. The disease is considered to exist in Angola, Burundi, Cameroon, Malawi, Mozambique, Uganda, Zaire and South Africa. There are claims that ASF has been eradicated from the islands of Sao Tome and Principe.

In the Western Hemisphere, free from the disease until the early seventies, ASF has been successfully eradicated from the Dominican Republic, Haiti and Cuba. The disease has not been reported in Brazil for several years and the Brazilian authorities indicate that they have been successful in eradicating ASF.

Hog Cholera

Hog cholera is causing increased concern in Europe because attempts to eradicate it are being intensified by the member countries of the European Economic Community. Whether such efforts lead to increased reporting of the disease or whether there is indeed a greater incidence is difficult to judge. Also, the classical signs usually associated with this disease have not been apparent, thus causing difficulty in recognizing the disease. The number of outbreaks reported from Belgium, Holland, and especially West
Germany have just begun to decrease. The disease is widespread throughout the world. Countries considered free of the disease this time include: Australia, Canada, Denmark, Dominican Republic, Finland, Great Britain (England, Scotland, Wales and the Isle of Man), Iceland, Northern Ireland, Republic of Ireland, Trust Territory of the Pacific Islands, New Zealand, and the United States of America.

**Swine Vesicular Disease (SVD)**

Not a single country reported Swine Vesicular Disease during this calendar year. However, the assumption that SVD may be disappearing might be too optimistic. Pirbright did identify SVD virus in August in material submitted from Hong Kong. USDA considers the following countries free of SVD: Australia, Bahama Islands, Canada, Central America, Panama, Haiti, Dominican Republic, Denmark, Sweden, Norway, Finland, Iceland, Greenland, Mexico, Northern Ireland, Republic of Ireland, New Zealand, Hungary, Yugoslavia, Bulgaria, Luxembourg, Romania, Switzerland, Trust Territory of the Pacific Islands, and the United States of America.

**Rinderpest**

Africa is sporadically reporting rinderpest. Fragmented local efforts to vaccinate seem to have prevented large scale outbreaks like those reported last year in Nigeria. Unfortunately, plans for a large regional campaign scheduled to begin in late 1984 had to be postponed for lack of financial support. This was to be similar to the “JP15” undertaking of the sixties. With the exception of cases reported from Persian Gulf ports where the disease appears among cattle imported under less stringent precautions, the existence of rinderpest in Asia is not well documented. Iraq had outbreaks in March. Iran claims to be free since the early eighties. Nepal had a problem in imported cattle and we have received unofficial reports that the disease may exist in North Korea.

**Contagious Bovine Pleuropneumonia**

In Africa, contagious bovine pleuropneumonia (CBPP) has been reported as endemic for certain areas of Namibia. Other countries in Africa have not reported CBPP this year. However, conditions in Africa frequently may preclude making disease reporting a high priority. There were no reports of this disease in Europe. In previous years, cases have been reported in Portugal, Spain and Southern France.

**Sheep and Goat Pox**

Sheep and goat pox is usually found in some of the countries surrounding the Mediterranean. In 1985, the disease was reported only from Morocco and Israel.

**Lumpy Skin Disease**

Lumpy skin disease is regularly reported from South Africa and from Madagascar. Sources in Somalia report increasing numbers of cases in the southern part of that country.
African Horse Sickness

African horse sickness is regularly reported from South Africa and occasionally from Namibia. In July 1985, a case was reported from Ghana.

Rift Valley Fever

Rift Valley Fever has not been reported since the outbreak of 1977 in the Nile Valley.

Glanders

Glanders was reported in South Africa and Turkey.

Dourine

Dourine is occasionally reported from South Africa, Namibia and Italy.

EMERGENCY PROGRAMS PROGRESS REPORT

During fiscal year (FY) 1985 (October 1, 1984, through September 30, 1985), there were 480 foreign animal disease diagnostic investigations. Of these, 440 cases were because of suspected vesicular conditions in cattle, horses, sheep, goats, and swine. Four hundred and thirteen of the investigations were made in the last quarter as a result of the recurrence of vesicular stomatitis (VS) type New Jersey in New Mexico, Arizona, and Colorado. The last significant outbreak in the United States took place in 1982–83 and was also the New Jersey type.

Vesicular stomatitis for the year started in December with one horse at Marfa, Texas, that was diagnosed positive for VS type New Jersey based on an increased titer and clinical signs compatible with VS.

The outbreak in three of the Southwestern States was first observed in New Mexico, with clinical signs suggestive of VS in horses and cattle during the middle of May. This was confirmed on June 10, 1985, by virus isolation at the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island, New York. The majority of the 64 cases in New Mexico were located in the Albuquerque area. VS in Arizona was first observed in mid-June near Ft. Huachuca, with serological confirmation completed the last of June. Isolation of VS virus from horses located at Pinetop, Arizona, was made on June 30. The first outbreak in Colorado was near Pueblo and was confirmed on July 17 by virus isolation. Colorado had 147 positive cases which occurred on both the eastern and western slopes. Most of the investigations were in the Pueblo area.

All states of the continental United States west of the Mississippi made investigations for possible vesicular conditions except Missouri and Nevada. Investigations were made in 10 states in the Eastern United States.

As a result of the VS outbreaks, two field studies were undertaken. One was to establish serological baselines for livestock in Colorado and New Mexico. Serums were obtained from laboratory, salebarn, and slaughter
1985 Vesicular Stomatitis Type New Jersey Outbreak in Three Western States:

<table>
<thead>
<tr>
<th>State</th>
<th>Investigation</th>
<th>Positive Virus</th>
<th>Positive Serology</th>
<th>Total</th>
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<tr>
<td>Colorado</td>
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<tr>
<td></td>
<td>104 bovine</td>
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<td>55</td>
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<tr>
<td></td>
<td>4 ovine</td>
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<td>2 caprine</td>
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<td></td>
<td>38 bovine</td>
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<tr>
<td></td>
<td>51 equine</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
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<td>64</td>
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</tbody>
</table>

Thirty-day State quarantines were recommended on all premises with positive animals. If fairs and shows could not be cancelled, officials in charge were encouraged to control insects with pesticides and observe other biosecurity measures.

During the investigation, epizootic hemorrhagic disease (EHD) was detected in cattle in Tennessee and Colorado.

Avian Influenza:

Eradication of H5N2 avian influenza in poultry was completed April 2, 1985. At last year's meeting, we reported that the area quarantines had been released in Virginia on September 14 and Pennsylvania on October 4, 1984, and that there remained a few Pennsylvania premises under quarantine for cleaning and disinfection completion. After release of quarantine, a 6-month surveillance program was carried out in each area and ended March 15, 1985, in Virginia and April 2, 1985, in Pennsylvania. During this surveillance period, two additional seropositive flocks were detected and depopulated in Pennsylvania. There were no seropositive cases in Virginia poultry nor any H5N2 virus detected in either state.

In December 1984, H5N2 avian influenza virus was isolated from chickens offered for sale on the streets of Washington, DC. The chickens and other poultry were seized from two people that were selling without a license. The virus was not pathogenic to poultry at the National Veterin-
ary Services Laboratories (NVSL), Ames, Iowa. Dr. R. G. Webster, St. Jude's Children's Research Hospital, reported the virus had similarities to the Pennsylvania virus as observed in April 1983. The dealer's premises and the two premises that received the seized poultry were depopulated. Two hundred and fifty backyard flocks in five states were investigated as a result of connections to the dealer. Results were negative.

**Tick Eradication:**

The program for *Boophilus microplus* and *Amblyomma variegatum* ticks in Puerto Rico is making substantial gains. Only three of the latter ticks were collected from cattle this year. The tick eradication program was further complicated by the diagnosis of babesiosis (cattle fever tick) in April, 1985. As of October 1, 258 premises having approximately 18,684 head of cattle are under quarantine for babesiosis in Puerto Rico. Babesiosis infected livestock are sprayed with a pesticide at 14-day intervals. The elimination of babesiosis is dependent upon the elimination of the *Boophilus* tick from Puerto Rico.

**Contagious Equine Metritis:**

While still in quarantine, a stallion imported from West Germany was found to be infected with contagious equine metritis (CEM). A great deal of treatment effort was necessary to rid him of the disease. After successful treatment, the animal was released from quarantine.

**Velogenic Viscerotropic Newcastle Disease (VVND):**

In October 1984, a pet bird shipped from California was found to be infected with VVND when examined in Hawaii. Epidemiological investigations failed to detect additional infection.

Between January 17 and February 25, 1985, pet birds were found to be infected with VVND on three Florida premises. Two premises were commercial wholesale facilities and the third was a private aviary. Tracing of sales from these premises disclosed an additional case in Puerto Rico. The tracing of birds to 25 states failed to detect additional cases.

In May, a privately owned pet bird in North Carolina was found to be infected with VVND. The infection was found at the bird's source, a Missouri dealer. Tracings from the dealer failed to detect additional cases.

**Nematodiasis:**

In February, 1985, a causative agent of nematodiasis, *Nematodirus battus*, was identified for the first time from several sheep in the Willamette River Valley in Oregon.

**Emergency Preparedness:**

Two foreign animal disease diagnostic training courses were conducted during the past year. We now have available in the United States a total of 260 veterinarians trained for field investigations of foreign animal diseases.

The APHIS four Regional Emergency Animal Disease Eradication
FOREIGN ANIMAL DISEASES

Organization (READEO's) are fully staffed to respond rapidly to outbreaks of emergency diseases.

APHIS Emergency Programs has continued cooperative activities with the Southeastern Cooperative Wildlife Disease Study in Athens, Georgia, and the University of Wisconsin, Madison. These projects are concerned with the role of wildlife in outbreaks of foreign animal diseases and for identification of VVND strains.

Emergency Programs staff has completed an Avian Influenza Eradication Guide. The Hog Cholera and Heartwater Eradication Guides will be available soon.

Emergency Programs Data Bank now contains about 54,000 articles on foreign animal diseases.

RESEARCH ON VESICULAR DISEASES

INTRODUCTION

This abbreviated review of research of the past year (1984–85) is focused upon those findings particularly relevant to viral vesicular disease of veterinary importance. These diseases are: Foot-and-Mouth Disease (FMD) [Picornaviridae, Aphthovirus (FMDV)]; Vesicular Stomatitis (VS) [Rhabdoviridae, Vesiculovirus (VSV)]; Swine Vesicular Disease (SVD) [Picornaviridae, Enterovirus (SVDV)]; Vesicular Exanthema of Swine (VES) [Caliciviridae, Calicivirus (VESV)].

Foot-and-Mouth Disease (FMD)

The half-life of colostrally acquired anti-FMDV antibodies in piglets is related to the time of antigenization of the sow in relation to farrowing. Doses of vaccine large enough to prevent viremia do not prevent FMDV replication in the respiratory tract. Cell lines persistently infected with FMDV have been described. Diagnostic and typing procedures for FMDV based upon ELISA are being developed. The FMD virion contains an endonuclease which destroys its infectivity rapidly at 37°C in the presence of suitable salts. Genomic basis for some variants was described. An enzymatically active FMDV specified protease was expressed in E. coli.

A biochemical map of the FMDV polypeptide was presented. Discontinuous epitopes related to antibody neutralization of FMDV infectivity were shown to exist on the virion and virion precursors but not on virion degradation products. Three neutralizing monoclonal antibodies each demonstrated different physicochemical phenomena associated in their reaction with FMDV: aggregation; interference with receptor binding; neither of these. Neutralizing antibodies specific for trypsin-sensitive sites were discussed and 2 epitopes involved in the neutralization of FMDV were precisely defined. Sensitive assays were used to study the anti-FMDV antibodies elicited with peptides and with whole virus.

The dosage of FMD vaccine affects the immediacy and duration of immunity as well as the magnitude of the anamnestic response. The neutralization reaction appears the most relevant FMDV typing pro-
procedure for purposes of selecting vaccine antigens. The humoral response plays the predominant role in the immunity of mice to experimental FMDV infection. Purification procedures are essential to the immunogenicity of FMDV peptides produced in E. Coli. Data on the dose response and long term protection elicited against FMD by genetically engineered peptides were reported.

**Vesicular Stomatitis (VSV)**

New members of the Vesiculovirus group have been described and their pathogenic and serologic characteristics are being investigated. For the most part nucleotide sequence data of the viruses examined is more similar than dissimilar and strongly indicate a common ancestor. Epidemiologic investigations show that the longer lived wildlife provide the most meaningful serologic data. Hybridization of RNA results show that VSV’s from horses and black flies (’82 CO) were essentially the same as VSV’s from other animals in that era.

The development and characterization of monoclonal antibodies may make possible certain simplifications in diagnostic procedures. For example, polyclonal antisera often may not distinguish NJ-VSV from IND-VSV; whereas, monoclonal antibodies can be developed which react with both serotypes (most likely directed to an epitope which is a poor eliciter of antibodies).

Cotton rats were protected from VS by aerosol treatment with biosynthetic alpha interferon (Human). Both Halothane and gamma interferon (Human) reduced VSV replication in tissue culture. Persistent VSV infections of tissue culture are being characterized. Recent investigations concerning the mechanisms of generation of DI particles as well as their influence as selecting factors during infections are being reported.

Signal “peptides” for transport (i.e. to the golgi, to the cell surface) and membrane anchoring are being studied in the VSV system. A multitude of reports on the physiologic roles of the VSV proteins have been published upon which progress in pathology at the molecular level can be based. Cell receptors for the endocytosis of VSV have been isolated and the characteristics of cell fusion due to VSV “G” protein are being investigated. Incorporation of host cell proteins into the VSV virion has been reported.

Vaccine made from virion protein “G” (from VSV) elicited protective immunity to VS in cattle and mice; however, synthetic peptides based upon protein “G” amino acid sequences were largely ineffective. A live vaccine composed of vaccinia virus carrying a genomic insert for VSV “G” protein elicited a measurable immune response. Basic research toward the development of viral vectors for vaccine is being done with VSV.

Numerous investigations of cell mediated immunity (CMI) mechanics have utilized VSV. Those directly relating the major histocompatibility complex (MHC) to particular antigenic determinants are important to
Swine Vesicular Disease (SVD)

Experiments were conducted at the Plum Island Animal Disease Center of the Agricultural Research Service, United States Department of Agriculture and at the Istituto Zooprofilattico, Brescia, Italy, to determine if the processing and curing of the Parma Hams would inactivate swine vesicular disease virus. The experimental data indicated that the virus was inactivated between 180 and 200 d post-slaughter in the U.S. experiment and between 90 and 182 d post-slaughter in the Italian experiment. The minimal curing period for Parma hams is 365 d, which would assure that the ham is free of infectious swine vesicular disease virus.

The transport of fresh and frozen semen for artificial insemination may transmit disease between farms. Many viruses, including swine vesicular disease virus have been detected in semen and have the ability to survive in frozen semen. However, the authors conclude the artificial insemination is less of a risk than the introduction of a boar into the herd.

In 1981/82, 26 outbreaks of swine vesicular disease were reported in the United Kingdom, 15 in Italy, 2 in the German Federal Republic and 1 in France. There have been no outbreaks of SVD in the UK since 1982 and serological surveys have been negative. One might assume that swine vesicular disease appears to be disappearing in Europe or at least the incidence of the disease appears to be greatly reduced.

Vesicular Exanthema of Swine (VES)

The caliciviridae contains Vesicular exanthema virus of swine, serotypes 1–12, San Miguel sea lion virus, serotypes 1–8, feline caliciviruses and possible calicivirus of calves and swine. Vesicular exanthema of swine was first recognized in 1932 and in 1959 declared eradicated and designated a foreign animal disease. Caliciviruses that could be classified as new serotypes of VESV have been isolated from marine mammals (seals) and from ocean fish.

Feline calicivirus infections in kittens borne by cats persistently infected with virus is reported by Johnson and Povey.

A new calicivirus isolated from a dog is described by Schaffer, et. al. This virus does not appear to be closely related to any previously described calicivirus except possibly the stunting syndrome agent of chickens.

Lesions in Gnotobiotic calves experimentally infected with a calicivirus-like (Newbury) agent are reported by Hall, et. al., from the Institute for Research on Animal Diseases, Compton, and the National Institute for Research in Dairying, Reading, England. The changes in the small intestinal structure and function were assessed qualitatively and quantitatively, by light microscopy, enzymology and Xylose absorption. There was individual variation in the severity of the effects of infection in calves killed at the same time after infection which appear to relate to the individual calf variation in the speed of the pathogenic process.
FOOT-AND-MOUTH DISEASE REFERENCES


FOREIGN ANIMAL DISEASES


VESICULAR STOMATITIS REFERENCES


SWINE VESICULAR DISEASE (SVD) REFERENCES

VESICULAR EXANTHEMA OF SWINE (VES) REFERENCES
MAINTAINING AND EXPANDING OUR SMALL RUMINANT EXPORT MARKET

by Robert K. Pelant, D.V.M.
Staff Veterinarian
Heifer Project International, Inc.
825 West 3rd St.
Little Rock, AR 72201

It is a distinct privilege and great pleasure to address the General Session of this convention.

During the past six months I have traveled through five continents for Heifer Project, observing and backstopping our programs. One overriding fact is that there is an ever-increasing use of small ruminants. And our programs respond directly to local demand.

Why the local demand?

The answer is partly found in the development policies of the foreign nations — their thrust for self-sufficiency in milk, meat and fiber production. It is also found in the availability of small ruminants. Sheer numbers of sheep and goats dwarf any other species of domesticated livestock. But the primary reasons for strong local demand are the adaptability, productivity and prolific nature of sheep and goats. Being ruminants, goats and sheep are able to convert lesser quality roughages into meat, milk and fiber. The bipedal stance of goats enables them to browse at higher levels. However, this survival technique, especially used during the latter stages of desertification (caused by climatological, socio-political and demographic variables) often earns them the incorrect label of destructive animals. At this stage however, most other animals are already dead.

Milk production based on body size is quite impressive in goats. Many thousands of families in foreign countries employ the services of the “poor man’s cow” to meet their daily milk needs.

Wool and hide production of small ruminants is a major reason for keeping them in many areas. Goat hides form a billion dollar a year industry in the People’s Republic of China. Hairsheep of the Katahdin breed, raised on our ranch in Arkansas have given large numbers of triplets and are performing well in rapid production of high quality meat in several Central American countries. The small size and propensity to have twins are other reasons why many farmers can and do keep small ruminants.

In other words, the local demand is based on all the abovementioned merits of small ruminants. And these merits are the very reasons why we can and should expand our exportation of sheep and goats from the United States.

We have the potential to respond to the great need of increased meat and milk production in goats and better meat and wool production in sheep. In the Heifer Project program: U.S. hairsheep are providing high quality
meat, and hair for weaving, to small farmers in Honduras; U.S. Nubian goats are upgrading herds in Cameroon, West Africa, for better milk and meat production; U.S. Hampshire rams are crossbreeding local ewes in the highlands of Peru to double body size and increase wool production 300%; U.S. Saanen goats are crossbreeding with local varieties to triple milk production and increase meat production by 75% in India, where cow meat is forbidden, but over half the population of 750,000,000 are not vegetarians. And the list goes on.

These farmers have chosen and depend on small ruminants for their very existence in many cases. The high quality stock from the United States can and should be used to facilitate this. By far, the greatest single market is the small farmer. They are certainly not the easiest to approach or coordinate, but their numbers make them a force to be dealt with if we are to appreciably increase our export of small ruminants.

Amidst all this positive potential exist some limited factors that if overcome will greatly facilitate the export of our small stock. There are difficulties we face with both long term and short term solutions.

Areas with more immediate solutions deal with relationships between APHIS and the industry, and the USDA and foreign health officials.

APHIS and the industry are working together in the joint USDA-Industry Livestock Export Task Force. The creation of species specific health certificates could only help the exportation of sheep and goats. The development of the computer based International Regulations Retrieval System (IRRS) will help clarify foreign health requirements, and facilitate their interpretation which will aid the export of all livestock. As the listings expand they should include more small ruminant health requirements which will reduce much of the bargaining and debating that now occurs between exporters and the importing country. Unfortunately, since small ruminants aren't as widely exported yet from the United States, foreign health officials do not often have well prepared, realistic requirements on hand and occasionally consult the OIE handbook.

A primary constraint to our export market with longer term solutions is the disease status of our small ruminants. Bluetongue and caprine arthritis are limiting our exports.

There are several options for exporters to consider when dealing with these conditions. The possibility of USDA-approved herd-free status certification for bluetongue is essentially nil at the present time. However, exporting animals raised in primarily bluetongue-free areas, will usually ensure less trouble at final test time. We have exported small ruminants from the northeast U.S. for many years without any major problem due to bluetongue. We are presently considering the potential benefits of routine herd testing for bluetongue. Caprine arthritis — both mycoplasmal and viral — is another constraint and potentially larger threat to the export market of goats. The establishment of official USDA herd free status certification for these types of caprine arthritis is also unlikely in the near future. However, the possibility of developing a herd free of mycoplasmal
and viral caprine arthritis is good. We raise goats in a closed herd, routinely testing all animals, pasteurizing colostrum and physically separating animals by age group. An important point is to do complete herd tests, and have this fact recorded on the test records. We have had a negative herd with regard to the viral arthritis test (AGID) for over one year, and we welcome the development of the newer more specific tests. I strongly believe that the Sheep and Goat Committee and the American Association of Sheep and Goat Practitioners should begin serious discussions concerning both arthritis free herds and ovine progressive pneumonia free herds. All rams exported for breeding purposes should be palpated for lesions of epididymitis, and have a complement fixation test for Brucella ovis.

Furthermore, species specific codes of welfare could be developed, perhaps along the lines of those published in Great Britain. This will increase the percentage of quality animals reaching their final destination, and will make U.S. stock more desirable to importers.

Thorough physical and performance examinations of individual animals exported for reasons other than immediate slaughter will ensure and help expand the market. Exportation as a sustained market effort would be greatly enhanced. We routinely employ more tests than required by the USDA for export, and vaccinate animals against the very common diseases with killed bacterins. In the case of live virus vaccines, a safety period is used to prevent shedding at an inopportune time. I have established health guidelines for each species we export, and am constantly upgrading them. Together with shipment guidelines and a checklist, we feel this aids us in providing reliable service and quality stock. I believe that to ensure a smooth operation from farm of origin to farm of destination, a set of procedural guidelines should be employed, and we welcome any development along these lines by APHIS.

We sent a livestock shipment preparation checklist to the primary responsible person on the receiving end before preparations are made for arrival and quarantine. A major area of concern in this regard is the type and quality of holding facilities available at foreign ports.

I believe that in dealing with importers, when no official health requirements are on hand from APHIS, as is often the case with small ruminants, it is wise to make a statement on the health status of the animals intended for shipment when soliciting health requirements. We provide a statement on health of the livestock to be shipped, and find that this usually results in more reasonable health requirements.

We believe that information provided by the importing country on the condition of the animals at arrival time, and any other problems that may have occurred is very helpful in assuring more successful and smoother subsequent shipments. In this regard, we provide shipment receipt and evaluation forms to the importers.

Finally, we wish to extend our sincere thanks to APHIS for all their help to our export program over the years. We have faith that through further
cooperation between the USDA and the industry, and development of sound export-oriented programs, both the quality of our small ruminants and their inherent merits will ensure an expanding and profitable export market well into the future.
SWINE EXPORT HEALTH CERTIFICATION

Dr. T. E. Socha
Executive Secretary — Nebraska SPF Export
Nebraska SPF Swine Accrediting Agency, Inc.

One of the U.S. swine industry's greatest blockades in the exportation of breeding swine is our own USDA and APHIS officials. My general experience has been that our bureaucratic system accomplishes those tasks that are most easily done. By this, I mean that the solution to any governmental request is one of putting additional requirements on the U.S. exporter rather than negotiating a realistic import specification list for other countries.

With those two opening statements, I want to give you some specifics of my experiences and then comment on possible changes in the USDA-APHIS methodology and maybe even their philosophies of animal export.

I have been personally involved in the preparation of animals for exportation to several countries, including a shipment to the Peoples Republic of China in 1982. I have also had an opportunity to discuss health matters with governmental officials from many foreign countries. I realize that our USDA-APHIS people get upset when anyone other than themselves talk to other governmental health officials, yet, my encounters lead me to believe that too often our own officials avoid having direct communications with foreign government health officials.

While in Beijing the Chinese Ministry of Agriculture asked to meet with our team to discuss swine health problems. First, I learned that they were unsuccessful in dealing through FAS for getting official meetings with our USDA people. Secondly, it soon was apparent that the FAS personnel were very limited in understanding swine health in our country or with PRC.

If anyone here is now convinced that I feel there should be a relaxation of requirements for exportation of swine, you are dead wrong. I have documented statements in my office to confirm the fact that we have attempted to send diseased animals to other countries. This list includes Mexico, Brazil, Argentina and at least four far eastern countries. My personal belief is that unless a foreign buyer specifically documents a list of requirements, our officials would allow any animal to be exported.

I cannot rationalize nor justify the existence of USDA health officials that feel we can adopt a buyer beware philosophy in the exporting of livestock. It is imperative that APHIS prepares a list of diseases that all exported animals be tested for. This list should include more than just diseases that can be checked for by a 30 day test.

We need to put some common sense into swine health. Perhaps one problem with this suggestion is that common sense is not a scientific method in dealing with health officials. But, I think we must be able to mix sound testing with practicality and not just sound testing.

Two examples of this lack of common sense are 1) we want pigs negative
of TGE titers for shipment, but, they must be vaccinated for TGE 15–30 days prior to shipment. Conscientious producers do not use vaccines for every imaginable disease in their herds. Plus, I have always felt that negative animals for export was our goal and not vaccinated animals. The second example of a failure to use common sense was a requirement to nasal swab pigs and test for bordetella as a measurement of AR in swine. Modern science and the highly educated APHIS officials have no logical explanation for this requirement, but, it is included in health protocols.

Testing methodology is another area of concern to most exporters. The APHIS guidelines suggest that specific tests be done for specific diseases. Yet one time we are told that the test must be done within 24 hours of collection of samples and later we find out that other people are able to collect the samples and send them by mail to a lab for testing 3–4 days later. Some labs find great joy in listing titers of any test without indicating a negative or positive reading; leaving the interpretation of these test results up to an area USDA-APHIS veterinarian or up to the buyer. Again an area of concern to producers attempting to comply with export requirements.

The next major obstacle in preparing animals for shipment is the quarantine facilities used in the U.S. For many countries, the animals must be isolated and kept separate from any other animals even though the tests required are only those done in the entire herd routinely and the herd is listed free of these diseases. Although this again shows the lack of common sense, it is not as important as the fact that after the quarantine, isolation and testing period, we are expected to deliver these same CLEAN animals to contaminated quarantine facilities. In some instances, the pigs not only need to use the U.S. quarantine facilities but also the importing country facilities that are contaminated with other swine.

APHIS needs to develop a method of inspection of animals in isolation that will then allow these animals to go directly to the designated freight carriers. I would also contend that the five hour inspection prior to loading is perhaps one additional stress that could be eliminated with proper inspections in the isolation units.

Changes in philosophy that I feel are needed in APHIS to aid and assist in the exportation of swine, are the following.

We need to identify those herds that are doing an excellent job of monitoring for specific diseases. Testing requirements in those herds should be different than in herds where no testing is done. I agree with all exporters that too often U.S. animals are considered to be carriers of all diseases. Some countries in Europe would be rather hostile if you assumed that all diseases were in all European countries. Likewise, FAS and APHIS need to acknowledge that there are differences in herd health in the U.S. swine industry.

We must as a country discontinue a policy of BUYER BEWARE and develop export requirements for all swine and perhaps all livestock. The contamination of other pig populations with our diseases is unjust and
perhaps immoral. Some countries would have fewer diseases of swine if it had not been for the diseased pigs sent there from the U.S.

USDA-APHIS and other governmental people must develop meaningful health protocols with other countries. Exporters need to know before contract negotiations what is expected and if the tests can even be accomplished in the U.S. These protocols should include quarantine handling of both countries. These protocol should also allow for differences as deemed suitable for the receiving country.

The USDA should develop a means of insuring payment for livestock exported to other countries if the animals have met all test requirements prior to shipment. To help with this, there should also be improved standardization of tests and test reports to aid in the determination of negative animals. I still believe that we should have one laboratory specifically for testing animals for export.

State-Area APHIS veterinarians should be knowledgeable on export requirements, but, much more, they should be there to help in the exportation of livestock and not as a barrier to hurdle. It amazes me that foreign buyers are more aware of what is happening in our swine industry than what our local APHIS personnel understand.

In summary, I want to re-emphasize the fact that I believe in more stringent export requirements, of all swine, than we presently have. I do not believe in the philosophy of exporting diseases with the animals.

With respect to APHIS, I think we need to develop better communications between APHIS offices, exporters and producers. By this I mean that APHIS should keep all segments informed of protocol changes and also that APHIS should be receptive to modify those protocols where inconsistencies exist, such as vaccination of negative animals and tests that are not valid for accomplishment of the goals.

I also feel that some national policies be developed where everyone can feel good about using U.S. breeding swine. Case in point in the Dominican Republic where an entire island was depopulated, but, due to APHIS recommendations and guidelines any U.S. swine could be imported to again give those people the opportunity to have all our diseases reintroduced.

I believe that the U.S. swine industry does have some of the best breeding swine available in this world. We have pigs that can gain 30 pounds per day, some that convert feed to gain at less than 2.0 and some herds that average more than 13 pigs born per litter. I also believe that we have the best health program anywhere in the world. After recently observing the Japanese SPF program and only last week discussing the Denmark SPF program, I'm certain that our SPF program is as good as any.

Therefore, I want to leave you with just a very simple thought. Since we do have genetically superior swine and since we do have excellent health programs, we could also be the leader in breeding swine export. To accom-
plish this goal we need everyone in the area of animal health to work towards realistic, practical, uniform testing protocols in export requirements. We have the livestock available, we only need the appropriate channels open for exporting.
REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: Clint Booth, Dallas, TX
Vice Chairman: Dan Childs, Lake Placid, FL

John Acree, CA; J. N. Armstrong, NV, W. A. Bailey, MD; C. T. Barnes, Jr., VA; J. L. Blair, VA; Ronald B. Caffey, MD; Tom Cook, DC; R. L. Evinger, TX; W. H. Fales, MO; Allan Furr, MD; W. B. Grene, FL; Frank Harding, IL; W. C. D. Hare, Canada; Rube Harrington, Jr., IA; David E. Herrick, MD; Michele C. Howard, CA; Harry W. Kinne, TX; R. C. Knowles, DE; Marline Main, SD; J. M. Massey, TX; Bob Mathis, AZ; J. W. McVicar, NY; M. E. Mix, VT; M. J. Nolan, DC; D. A. Price, CO; W. D. Prichard, OR; Glenn B. Rea, OR; Charles Reid, FL; T. D. Rich, MO; James D. Rosswurm, CA; Robert Rumler, VT; S. V. Timberlake, Jr., NY; J. S. Walker, DC; H. A. Waters, VA; Carl Weston, NH; Walker Wilson, TX; George O. Winegar, MD; R. J. Yedloutschnig, NY

The Committee on Import-Export met on October 30, 1985, during the annual meeting of the USAHA held at the Marc Plaza Hotel, Milwaukee, Wisconsin. The meeting was called to order by Chairman Booth with 27 members present and with a total attendance of 45 people.

The Chairman asked the committee for comments on last year's report and no comments were offered.

Dr. R. B. Caffey reviewed the past year's activity in Plant Protection and Quarantine and Dr. D. E. Herrick, assisted by Drs. G. O. Winegar and Allan Furr, reviewed the past year's activity by the Import-Export Program of Veterinary Services. Their reports are attached as Appendix 1.

Dr. R. B. Caffey showed the committee a videotape on passenger baggage inspection using passively trained dogs. These clips from national television were evidence of the good public relations this program is generating. It has been very successful with teams at four airports. There will be 12 teams by the end of 1986 and eventually about 25 teams operating at our international airports.

Dr. D. W. Luchsinger from Plum Island presented a paper "Report on Cell Line Testing Program at the FADDL" by Drs. C. House, R. J. Yedloutschnig and J. A. House which is attached as Appendix 2.

Mr. S. V. Timberlake, Jr., assisted by Drs. D. E. Herrick and G. D. Winegar, gave a report of the Embryo Movement subcommittee meeting that was held on Tuesday morning (attached as Appendix 3).

Dr. H. A. Waters presented a report of the Export subcommittee meeting which was held on Tuesday afternoon (attached as Appendix 4).

The committee considered and passed the following resolutions:

1. International Embryo Movement — which urges the USDA APHIS to sponsor directly or indirectly a symposium to exchange information on embryo transfer.

2. Zoological Animals — endorsing a resolution from the zoological
animals committee that would permit the release of U.S. origin animals from zoos if they had not been in direct contact with permanent postentry quarantine animals and were free of clinical signs of disease.

3. Imported Mexican Cattle — endorsing a resolution from the Tuberculosis committee that would require these cattle to be quarantined and tuberculosis tested by U.S. officials.

The committee adjourned at 5:30 p.m.

APPENDIX 1

APHIS Report to the Import-Export Committee of USAHA

Civil Penalties

Civil penalties assessed against travelers at land border, air, and sea ports appear to have enhanced compliance with animal product entry requirements. During the 6-month period April 1 to September 30, 1984, 10,895 civil penalties were assessed. During the same period in 1985, 8,676 penalties were assessed which represents a 20 percent reduction. During all of FY 1985, 14,966 penalties were assessed and 426,559 dollars collected.

In addition to the civil penalty program on passenger baggage which was initiated nationwide in March 1984, a similar program relative to maritime garbage handling violations was implemented July 1, 1985. Between July 1 and October 10, 220 violations were found by Plant Protection and Quarantine Officers. These resulted in the collection of $20,150 in penalties. Unfortunately, 71 violations could not be processed due to a technical deficiency in the existing regulations requiring the government to prove that garbage held in violation of 9 CFR 94.5 requirements is derived from food items of foreign origin or that have been in a foreign port. When a vessel has purchased U.S. stores after arrival from a foreign port, legal satisfaction of these requirements is very difficult.

It was originally planned to amend the garbage regulations to alleviate the legal technicality by July 1. However, the necessary legal work has not been accomplished and we are unable to prosecute approximately one-third of the known violators.

Detector Dogs

Guidelines are being finalized for expansion of the detector dog program. At least 12 new teams will be trained and placed during FY 1986.

"Teams" are presently working at four international airports, John F. Kennedy International, Houston Intercontinental, Los Angeles International, and San Francisco International Airport. Public acceptance of passively trained beagles continues to be very favorable.
Fiscal Year 1985 Report of Animal Products Imported/Exported
(Does not include months of August and September)

Vessel and aircraft arrivals
37,851 vessels boarded
2,105 lots consisting of 517,359 kilograms of garbage were removed from these vessels
1,178 garbage violations/discrepencies
228,757 aircraft arrived from foreign locations
34,377,395 kilograms of garbage removed from these aircraft

Meat and other animal products confiscated/refused entry
ship passenger baggage 509 lots 10,039 kilograms
aircraft passenger baggage 92,081 lots 260,057 kilograms
border crossing 23,748 lots 35,913 kilograms
post office 5,253 lots 9,982 kilograms

Commercial poultry and red meat shipments rejected
344 lots 533,328 kilograms

Footwear cleaned and disinfected 2,314 pair

Animal by-product certificates issued 15,489

Commercial animal products imported
Restricted entry 3,899 lots 69,009,700 kilograms
Non-restricted entry 23,832 lots 954,862,282 kilograms
Refused entry 501 lots 8,680,260 kilograms

Animal Products and Byproducts
During fiscal year 1985, 796 permits were issued authorizing the importation of restricted animal products/byproducts and controlled materials for commercial distribution. During the same time period 1,197 permits were issued for the importation of controlled biological materials for research purposes including permits authorizing the import and interstate movement of animal disease organisms and vectors. The total number of import permits from the Animal Products/Organisms and Vectors sections (1,993) represents a 25 percent increase from the number of permits issued (1,590) in FY 1984. Some of the reasons for this increased interest in importing restricted and controlled materials are a strong U.S. dollar which allows more buying power abroad and increased interest from U.S. biotechnology companies to bring in cell lines, monoclonal antibodies, and other biologicals for this rapidly expanding industry.

Undercooked Hungarian Pork
Veterinary Services (VS) was notified on December 1, 1984, that a shipment of perishable canned hams from Hungarian establishment No. 6
failed the phosphatase test for thoroughness of cooking. Future shipments were sampled at a much higher sampling rate; however, there were no further reports of undercooking. The Animal and Plant Health Inspection Services (APHIS) requires that cooked pork products from countries where foot-and-mouth disease (FMD), swine vesicular disease (SVD), or hog cholera (HC) exists be cooked to a minimum internal temperature of at least 156°F to ensure destruction of these exotic viruses.

Changes in Disease Status for Great Britain, Norway, and Belgium

Great Britain was removed from the list of countries designated as being affected with SVD and viscerotropic velogenic Newcastle disease on February 19, 1985. The Department recognized Norway as being free of hog cholera on April 29, 1985. On March 12, 1985, Belgium was added to the list of countries where African swine fever (ASF) exists.

Completion of Virus Survival Studies on Prosciutto

Researchers at the Plum Island Animal Disease Center have completed virus survival studies in prosciutto, utilizing hams from pigs inoculated with FMD, HC, SVD, and ASF. Their findings indicated that none of the viruses are capable of surviving 400 days of curing/drying.

APHIS is considering publication of a proposal to allow the importation of prosciutto from Italy (and other countries where ASF exists) provided the hams are produced in VS-approved plants in strict accordance with the processing and recordkeeping requirements described in the proposed rulemaking.

Importation of Cooked Pork and Pork Products From Countries Where ASF Exists

The U.S. Department of Agriculture (USDA), APHIS, VS, published a proposed rulemaking to allow the importation of cooked pork and pork products from countries where ASF exists, provided the approved overseas processing establishments utilize only pork from ASF-free countries and provided the pork was cooked to at least 156°F throughout. VS received 11 comments in favor of the proposal and 7 comments against the proposal. A final decision on the docket is pending.

Processing Plants in West Germany Producing Cured/Dried Pork Products for Export to the United States

A VS representative in Europe has recently concluded a review of all of the West German meat processing establishments that produce cured/dried pork products for export to the United States to determine if they are in compliance with Section 94.12 of Title 9, Code of Federal Regulations. Specifically, 94.12(b)(iv) requires that the establishment use only pork or pork products which originate from countries recognized by USDA as being free of SVD. USDA does not recognize West Germany as SVD free. Preliminary findings indicates none of the West German plants are in compliance. A final decision will be made after VS has received and evaluated the written report.
Revised Regulations Governing the Importation of Cheese from Countries Not Declared Free of FMD

Countries not recognized as being free of FMD must now comply with new APHIS regulations (Section 94.16, Title 9, Code of Federal Regulations) governing the importation of cheese containing meat, liquid milk, and/or cream if these ingredients were added to the cheese after fermentation. Under the provision of these new requirements, U.S. importers must apply for a USDA permit in order to import cheese that contains meat, fresh milk, or cream. USDA permits are issued only if the meat or added milk/cream was processed in a manner sufficient to destroy the virus causing FMD.

Revised Policy for the Importation of Cell Lines Including Hybridomas

VS has revised the requirements for safety testing of cell lines, including hybridomas, imported from countries not recognized by USDA as being free of FMD. These revised procedures have expedited import of the material to the Foreign Animal Disease Diagnostic Laboratory and reduced the costs for safety tests. Copies of this VS Notice on the Importation of Cell Lines (dated June 25, 1985) may be obtained directly from USDA, APHIS, VS, Import-Export Animals and Products Staff, 6505 Belcrest Road, Hyattsville, MD 20782.

There is still an increasing number of immediate slaughter swine being imported from Canada. It is estimated that there will be approximately a 50 percent increase over 1984. From 1982 to 1984, the number of swine imported has doubled each year. This is attributed to a strong U.S. dollar in relationship to the Canadian dollar, a more favorable market in the U.S., the closing of several slaughter plants in Canada, and the subsidy that the swine producer in Canada had received.

A total of 90 head of cattle were released in March of 1984 from the Harry S Truman Animal Import Center (HSTAIC) at Key West, Florida. These cattle originated in France, Switzerland, Italy, and West Germany. One animal from Great Britain that had been born in France was returned to the Brest, France quarantine station for importation through HSTAIC.

A combination of factors discouraged potential importers from applying for sufficient numbers of animals to make an importation of animals economically practical for this year.

A review of the procedures was made in May 1985 in an effort to simplify the application process and reduce costs of importing while still maintaining adequate safeguards against the introduction of disease.

Regulations were initiated to relieve some of the contagious equine metritis (CEM) testing requirements for mares that are certified to be in racing or training status by the registry in countries that maintain such a registry.

A shipment of 65 zoo animals was imported in December of 1984 from
Kenya, Africa. This shipment included 21 giraffes. This was the first large shipment of giraffes imported in over 12 years.

Regulation changes are in process to add elephants, hippopotami, rhinoceros, and tapir to the animals that will require specific inspection for ectoparasites at the importation station.

Eleven countries have submitted requests and documentation to be recognized free of specific disease. The following table summarizes the requests and status of these countries.

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>DISEASE</th>
<th>REQUEST STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norway</td>
<td>Hog Cholera</td>
<td>Granted</td>
</tr>
<tr>
<td>Belgium</td>
<td>CEM</td>
<td>Denied</td>
</tr>
<tr>
<td>St. Lucia</td>
<td>VVND</td>
<td>Denied</td>
</tr>
<tr>
<td>Panama</td>
<td>Hog Cholera</td>
<td>Pending — more information</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>FMD (3 requests)</td>
<td>Denied</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>FMD</td>
<td>Denied</td>
</tr>
<tr>
<td>Chile</td>
<td>FMD</td>
<td>Pending — more information</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>SVD</td>
<td>Granted</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>VVND</td>
<td>Granted</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>ASF</td>
<td>Granted</td>
</tr>
<tr>
<td>Romania</td>
<td>FMD</td>
<td>Denied</td>
</tr>
</tbody>
</table>

Eleven states (California, Colorado, Kentucky, Louisiana, Maryland, New York, North Carolina, Ohio, South Carolina, Tennessee, and Virginia) have been approved to complete the treatment and testing of stallions for CEM. Wisconsin has requested to be approved. Eight states (California, Colorado, Kentucky, Louisiana, New York, South Carolina, Tennessee, and Virginia) have been approved for the treatment and testing of mares for CEM. Wisconsin and Maryland have requested to be approved.

The veterinary colleges at Ithaca, New York, and Davis, California, are approved to do corrective surgery for incomplete sinusectomies.

A group of swine industry and research personnel met with the staff to discuss the protocols and requirements to import germplasm from the People's Republic of China.

The dockets to import semen and embryos from countries not affected with foot-and-mouth disease are being reviewed by the Office of the General Counsel (OGC) and the Office of Management and Budget (OMB).

**ANIMALS IMPORTED**

<table>
<thead>
<tr>
<th></th>
<th>FY 1982</th>
<th>FY 1983</th>
<th>FY 1984</th>
<th>FY 1985 (Estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>804,435</td>
<td>1,224,076</td>
<td>787,724</td>
<td>532,443</td>
</tr>
<tr>
<td>Swine</td>
<td>224,004</td>
<td>416,224</td>
<td>1,066,056</td>
<td>1,481,984</td>
</tr>
<tr>
<td>Horses</td>
<td>32,398</td>
<td>36,232</td>
<td>35,776</td>
<td>29,629</td>
</tr>
<tr>
<td>Sheep</td>
<td>8,968</td>
<td>9,980</td>
<td>13,362</td>
<td>9,540</td>
</tr>
<tr>
<td>Others</td>
<td>15,061</td>
<td>3,494</td>
<td>8,419</td>
<td>8,320</td>
</tr>
<tr>
<td>Total</td>
<td>1,084,866</td>
<td>1,690,006</td>
<td>1,911,337</td>
<td>2,061,916</td>
</tr>
</tbody>
</table>
CATTLE

Canadian Ports
Air & Ocean Ports
Mexican Ports
Total

475,193
171
329,071
804,435

457,550
150
766,376
1,224,076

348,982
221
438,521
787,724

351,600
110
180,733
532,443

SWINE

Canadian Ports
Air & Ocean Ports
Mexican Ports
Total

223,533
471
416,224
224,004

416,056
168
1,066,056
1,481,984

1,066,014
42
416,056
1,481,984

1,481,845
137

HORSES

Canadian Ports
Air & Ocean Ports
Mexican Ports
Total

25,840
4,317
2,241
32,398

27,227
4,536
4,469
36,232

27,395
5,175
3,206
35,776

22,450
5,119
2,060
29,629

Construction

Sweetgrass Montana Border Facility — Construction for the facility started in late August and should be completed by the end of fiscal year 1986. Customs has agreed to provide the department with $35,000 for the construction of a cattle scale to be added to the facility. They will provide an additional $40,000 next year for the construction of an addition to house the scale.

Detroit Michigan Border Facility — This facility is still in the design and planning stages. The site has not been approved as yet, however, we plan to make a decision this fiscal year.

Export Animals

The import health requirements of other countries have been placed in the International Regulations Retrieval System (IRRS) which is located at the National Center for Animal Health Information Systems, Ft. Collins, Colorado. The VS area offices that have Brucellosis Information System (BIS) capability will have access to the IRRS. Changes in the import requirements of other countries can be more rapidly sent to all stations by this system.
A large number of the memoranda of the 592 series were updated this year and several new memoranda were written.

The 2nd and 3rd shipments of cattle exported, directly from the U.S. to Australia, totaling 210 animals, were shipped during this fiscal year. The fourth shipment, containing 96 animals has been in preembarkation isolation at Grays Lake, Illinois since the last week of September 1985.

A shipment of 330 swine from Illinois to Venezuela died of heat prostration at Caracas International Airport. Mechanical difficulties in the nose landing gear of the aircraft caused a belly landing of the plane. There was a two hour delay in transferring the swine because of the danger of explosion and the swine had no ventilation for controlling heat.

The United States was declared free of lethal avian influenza (AI) this year. Taiwan still will not accept poultry and poultry products from a state where AI has occurred during the past 12 months. Chile still does not accept poultry from Pennsylvania, New Jersey, Maryland, and Virginia due to the AI outbreak.

The Livestock Export Task Force met three times during this fiscal year. Leadership of the group is in the private sector with VS participating in an advisory role. Work will continue to make changes that will improve the quality of U.S. export livestock through cooperation by industry as a result of the liaison between industry and government in the Task Force.

A meeting with Canadian animal health officials in Ottawa, Canada, resulted in the rewriting of memorandum number 592.1, import health requirements for domestic animals exported from the United States to Canada. The requirement that donor dams of bovine embryos be tested twice for bluetongue was reduced to one test. The proposed requirement for isolation of feeder steers and spayed heifers was also amended.

A new animal health agreement was signed on January 17, 1985, with Egyptian animal health officials for export of slaughter and feeder cattle to Egypt.

The Export Staff met on April 17, 1985, with Dr. Teruhide Fujita, Deputy Director, Animal Health Division, MAFF, Japan. Discussions were held on export of U.S. bovine and swine semen, bovine embryos, and poultry. A new health certificate specific for bovine semen was agreed upon.

A meeting with British veterinary officials resulted in several changes in the animal health protocol for U.S. bovine semen and embryos to England for the 1985–86 production season. Three artificial insemination (AI) centers produced bovine semen for export to Great Britain in the 1984–85 season. The Export Staff reviewed export activities at the Texas and Minneapolis area offices. Only a few minor corrections in procedures were recommended.

The first shipments of U.S. horses to Thailand and Malaysia, swine to Czechoslovakia and Cyprus, cattle and horses to Pakistan, and bovine semen to Japan, were made during the fiscal year.
Seven (7) shipments of cattle totaling 1,197 head were exported to the People’s republic of China (PRC) in 1985. The cattle were tested to meet the requirements of protocol signed in May 1984. Testing had to take place between November 1 and April 15. The animals originated in the 18 northeastern states and western Washington. On arrival in the PRC, they were placed in quarantine for another 45 days and retested. The extensive testing and handling required by the protocol has discouraged many exporters. Efforts are being made to further amend the protocol prior to the 1986 export season.

Three shipments of swine to the PRC were also made during the fiscal year. The very demanding protocol has kept exports at a low level.

Approximately 93,000 day-old chicks were exported to the PRC in the fiscal year and indications are that the number will increase in fiscal year 1986.

Negotiations with Dutch Veterinary Authorities resulted in a change of the protocol for bovine embryos exported from the U.S. to the Netherlands. The protocol was made less restrictive by deleting the bluetongue and leukosis testing requirements for donor dams of the embryos.

The tripartite countries (England, France, and Ireland) changed their import health requirements for horses from the U.S. because of equine viral arteritis (EVA). All horses must now be isolated in a USDA approved isolation facility for 30 days before exportation.

Vesicular stomatitis was diagnosed again this fiscal year in Colorado, Wyoming, Texas, and New Mexico. Taiwan will not accept livestock from states where there has been a case of vesicular stomatitis during the last 12 months.

The Government of Venezuela is amending their import requirements for U.S. livestock. They have submitted a draft proposal of the requirements for cattle exports for VS comment and will submit drafts for other species by November 1, 1985. The new requirements will take effect March 1, 1986. A bluetongue test will be one of the additional requirements.

The recent diagnosis of canine ehrlichiosis in Hawaii will affect exportation of dogs to Australia. Previously all dogs exported to Australia from the U.S. were quarantined for six months in Hawaii because it is rabies-free.

The Government of Italy changes its import requirements for U.S. bovine semen. The semen can only be collected during the bluetongue non-vector season. If the results of the bluetongue survey in the northern states are satisfactory, semen collection will be allowed on a year round basis in the low incidence areas.
REPORT OF THE COMMITTEE

FISCAL YEAR ENDING SEPTEMBER 30, 1984

<table>
<thead>
<tr>
<th></th>
<th>CANADA</th>
<th>MEXICO</th>
<th>OTHER</th>
<th>TOTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>13,034</td>
<td>89,205</td>
<td>18,218</td>
<td>120,457</td>
</tr>
<tr>
<td>Horses</td>
<td>6,287*</td>
<td>1,204</td>
<td>2,517</td>
<td>10,008</td>
</tr>
<tr>
<td>Sheep/Goats</td>
<td>21,998</td>
<td>361,743</td>
<td>1,973</td>
<td>385,714</td>
</tr>
<tr>
<td>Swine</td>
<td>1,268</td>
<td>4,160</td>
<td>12,913</td>
<td>18,341</td>
</tr>
<tr>
<td>Total Livestock</td>
<td>42,587</td>
<td>456,312</td>
<td>35,621</td>
<td>534,520</td>
</tr>
</tbody>
</table>

Poultry (Live)

(a) Baby Chicks | 11,872,960 | 1,815,643 | 13,236,397 | 26,925,000 |
(b) Turkey poults | 614,878    | 102,822   | 149,300   | 867,000   |
(c) Other poultry | 2,111,215  | 149,500   | 314,285   | 2,575,000 |
Total Poultry    | 14,599,053 | 2,067,965 | 13,699,982 | 30,367,000 |

Hatching eggs (doz.) | 6,263,123 | 130,338   | 6,120,565 | 13,114,000 |

Bull Semen (dollar value) | 776,818    | 1,951,985 | 19,999,370 | 22,728,173 |

Data does not include horses exported to Canada and returned within 72 hours.

Certificates were issued which would include exhibition and race horses that reenter the United States indicating a total export of horses of 47,237 head.

11 MONTHS ENDING AUGUST 31, 1985

<table>
<thead>
<tr>
<th></th>
<th>CANADA</th>
<th>MEXICO</th>
<th>OTHER</th>
<th>TOTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>12,540</td>
<td>84,098</td>
<td>17,419</td>
<td>114,057</td>
</tr>
<tr>
<td>Horses**</td>
<td>6,077*</td>
<td>1,106</td>
<td>2,387</td>
<td>9,570</td>
</tr>
<tr>
<td>Sheep/Goats</td>
<td>21,934</td>
<td>341,728</td>
<td>1,928</td>
<td>365,590</td>
</tr>
<tr>
<td>Swine</td>
<td>1,268</td>
<td>4,405</td>
<td>11,396</td>
<td>16,709</td>
</tr>
<tr>
<td>Total Livestock</td>
<td>41,819</td>
<td>430,977</td>
<td>33,130</td>
<td>505,926</td>
</tr>
</tbody>
</table>

Poultry (Live)

(a) Baby Chicks | 11,311,793 | 1,582,069 | 11,740,626 | 24,634,488 |
(b) Turkey poults | 527,040    | 102,822   | 126,545   | 756,407   |
(c) Other poultry | 1,985,961  | 490,500   | 61,237    | 2,415,224 |
Total Poultry    | 13,824,794 | 1,834,391 | 12,146,934 | 27,806,119 |

Hatching eggs (doz.) | 5,776,417 | 125,900   | 6,120,565 | 12,022,882 |

Bull Semen (dollar value) | 776,818    | 1,752,322 | 18,697,234 | 21,216,374 |

*Data does not include horses exported to Canada and returned within 72 hours.
Avian Import Activities

A. Commercial Bird Program

1. There were twenty velogenic viscerotropic Newcastle disease (VVND) and velogenic Newcastle disease (VND) infected commercial bird lots from thirteen countries. The countries were: Netherlands, Peru, Argentina, El Salvador, Honduras, India, Singapore, Belgium, West Germany and Guyana. These birds were refused entry into the United States, no VND or VVND was introduced into the United States from legally imported birds since 1974.

2. Approximately 575,000 commercial birds were imported through eighty-four USDA supervised and three operated animal import centers. This is a decrease of almost 200,000 from FY 1984. This includes 55,000 birds, in 20 lots, which were refused entry into the United States because of VVND virus isolations.

3. Detroit, Michigan should be closed to commercial bird imports this year. Detroit bird stations will move to Miami, Florida. Six from New Orleans and one from San Diego will also move to Miami, during 1986.

4. A proposal to set up a closed commercial bird breeding colony of U.S. origin birds outside the United States, was withdrawn because of possible poultry disease exposure. Birds from the colony would enter the United States without quarantine.

5. Action has been initiated to remove the USDA approval to operate several privately owned quarantine stations in California, New York, and Hawaii, because of nonuse and failure to maintain USDA standards for approved facilities.

B. Pet Bird Program

1. U.S. Public Health Service deregulated the importation of psittacine birds July, 1985. Time and budget constraints forced them to reduce programs of lesser human health significance such as psittacosis.

   a. USDA will continue to feed chlortetracycline to all psittacine birds in quarantine to control psittacosis.

   b. Also pet bird owners are no longer limited to importing two psittacines per family per year.

2. There was one VVND isolation in a personal pet bird in the Miami quarantine station.

C. Smuggled Bird Program

1. The new permanent smuggled bird station in Otay Mesa, California, is operating.

2. There were seven VVND isolations from seven seized lots of birds in
D. Poultry and Hatching Egg Program

1. Poultry hatching egg imports for FY 1985 were 4,938,978. Baby Chick Imports for FY 1985 were 3,191,705.

2. In FY 1986 there will be a personal change Title 9, Code of Federal Regulations, Part 92.11(c), Poultry Quarantine Requirements, to discontinue quarantine of poultry hatching eggs from VVND-free countries. Hatching eggs from VVND-free countries have not been quarantined since the early 1970's. Their flocks of origin are tested negative for pullorum-typhoid disease and Adenovirus 127 (egg drop syndrome). Duck and geese hatching egg flocks of origin must test negative to Derszy's disease.

APPENDIX 2

REPORT ON CELL LINE TESTING PROGRAM AT THE FADDL

C. House, R. J. Yedloutschnig and J. A. House

Presentation at the U.S. Animal Health Association Import/Export Committee October 1985

In 1983, an ad hoc committee was appointed within the National Veterinary Services Laboratories to provide recommendations for safety testing of imported cell lines for foreign animal disease agents that would achieve a high degree of assurance while minimizing costs. These recommendations have evolved into protocols which have been in use since October 1984. Applications have been received for the testing of 55 cell lines in the 8-month period of October 1, 1984, to May 31, 1985.

Briefly, the process begins with the importer applying for a permit from the Import/Export Animals and Products Staff of Veterinary Services (Table 1). A cost estimate is generated by the staff at the Foreign Animal Disease Diagnostic Laboratory (FADDL) based on the diseases present in the country of origin, the laboratory of origin, and source of the culture's components. The 2 indicates the disease agents, their occurrence, and the test system used to detect the viruses. For example, in Table 3, a cell line from Australia would be tested for bluetongue, akabane, and bovine ephemeral fever viruses. A cell line from Japan, cultivated with bovine serum from Australia, would be tested for the same viruses. If porcine trypsin prepared in Europe were used in the cell lines passage history, tests for swine vesicular disease virus (SVDV), foot-and-mouth disease virus (FMDV), and hog cholera virus (HCV) would be added. European cell lines are tested for diseases present in their country of origin (Table 4) as well.

The average cost estimate for testing cell lines from Japan and Australia, using indigenous bovine serum, has been approximately $350 per cell line. The average cost estimate for European origin cell lines has been
approximately $2,000 per cell line due to the cost of cattle used in the FMDV test. To date, no application for cell lines originating from South America has been made. The cost estimates would probably exceed $2,350. (Table 5).

Concurrently, a USDA permit is used for the importer to ship the materials to FADDL. This permit, valid for 6 months, is also used to ship the tested cell lines to the importer. Sufficient ampules should be provided for testing and for final receipt by the importer. A minimum of four ampules containing a total of 4 ml and four million cells is usually required for each safety test. Ampules for final delivery are held in storage and are shipped to the importer following completion of the safety test.

Upon receipt at the Plum Island Animal Disease Center (PIADC), the ampules of cells are unpacked in a biological safety cabinet located in a special laboratory physically remote from the research and diagnostic laboratories. Samples are taken for testing, and the remaining materials are replaced and held in the gaseous phase of liquid nitrogen in cryogenic storage units. Usually, testing requires approximately 6-8 weeks, depending on the number of test procedures.

Test systems often include the serial passaging of the material in an exquisitely sensitive cell culture system and examination of the final passage by specific fluorescent antibody tests. The identification of bluetongue virus, bovine ephemeral fever, African swine fever, and hog cholera viruses are examples. Animal systems include the isolation of mouse lethal agents such as adabane and bovine ephemeral fever viruses by intracranial inoculation of 1-2 day old mice and isolation of FMDV by intradermalingual inoculation of steers.

From October 1, 1984, to May 31, 1985, requests have been received to test 55 cell lines (Table 6). Mouse hybridoma cells (41 cell lines) are the largest single category with the majority of these cell lines coming from Australia (17 cell lines) or Japan (12 cell lines). The present high cost of testing European origin cells may account for this distribution. Private industry is the recipient of 58 percent of these 55 cell lines with Australia and Europe being the leading exporters to private industry. Commercial distributors of cell lines imported 35 percent of the cell lines, mostly from Japan. Teaching and research facilities received only 7 percent of the cell lines, roughly equal from Eastern and Western sources.

The 20 cell lines safety tested during this period have been free of the adventitious agents for which they were tested. Receipt of the remaining 35 cell lines is expected between June 1, 1985, and November 15, 1985.

REFERENCES

3. FADDL Protocol 610: Bluetongue (BLU) and epizootic hemorrhagic disease


Table 1. Sequence of Events for cell Lines Entering the U.S.A.

<table>
<thead>
<tr>
<th>Process</th>
<th>Approximate Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Application reviewed</td>
<td>Varies</td>
</tr>
<tr>
<td>II. Permit issued &amp; costs estimated.</td>
<td>2 weeks</td>
</tr>
<tr>
<td>III. Cell lines received, sampled and tested</td>
<td>6 to 8 weeks</td>
</tr>
<tr>
<td>IV. Cell lines released</td>
<td>1 week</td>
</tr>
</tbody>
</table>
### Table 2. Disease Agents Possibly Present in Cell Cultures: Methods of Testing

<table>
<thead>
<tr>
<th>Agent</th>
<th>Classification</th>
<th>Occurrence</th>
<th>Test System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease (EHD) virus</td>
<td>Orbiviruses</td>
<td>Africa, Middle East, Asia, Iberia, N&amp;S America, Australia</td>
<td>3 blind passages: Vero cell cultures and suckling mouse brain (3)</td>
</tr>
<tr>
<td>Akabane Virus (AKA)</td>
<td>Arbovirus</td>
<td>Australia, Japan, S. Africa, Africa, Middle East</td>
<td>3 blind passages: Vero cell cultures and suckling mouse brain (4)</td>
</tr>
<tr>
<td>Bovine Ephemeral Fever Virus (BEFV)</td>
<td>Lyssavirus</td>
<td>Africa, Asia, Australia, Japan</td>
<td>3 blind passages: Vero cell cultures and suckling mouse brain</td>
</tr>
<tr>
<td>Foot-and-Mouth Disease Virus (FMDV)</td>
<td>Aphthovirus</td>
<td>Worldwide, except for Central &amp; N. America, Japan Australia, certain Caribbean islands, Great Britain, Ireland, North Ireland</td>
<td>3 blind passages: PK15 cell culture (6)</td>
</tr>
<tr>
<td>Hog Cholera Virus (HCV)</td>
<td>Pestivirus</td>
<td>Worldwide, except for N. America, Australia, New Zealand, certain European Countries</td>
<td>3 blind passages: IBRS-2 (D10) cell cultures (7)</td>
</tr>
<tr>
<td>Swine Vesicular Disease Virus (SVDV)</td>
<td>Enterovirus</td>
<td>Worldwide except Western Hemisphere, Australia, New Zealand, Africa</td>
<td>3 blind passages: swine buffy coat cultures (8)</td>
</tr>
<tr>
<td>African Swine Fever Virus (ASFV)</td>
<td>Iridovirus</td>
<td>Europe, Africa</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Possible Disease Agents in Australian and Japanese Origin Cell Cultures

<table>
<thead>
<tr>
<th>Country</th>
<th>Disease</th>
<th>AKA*</th>
<th>BT*</th>
<th>BEF*</th>
<th>EHD*</th>
<th>HC**</th>
<th>SVD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Japan</td>
<td>Yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

*Fetal bovine serum is possible source.
**Porcine trypsin is possible source.

### Table 4. Possible Disease Agents in European Origin Cell Cultures

<table>
<thead>
<tr>
<th>Country</th>
<th>Disease</th>
<th>ASF*</th>
<th>BT*</th>
<th>EHD*</th>
<th>FMD*,**</th>
<th>HC**</th>
<th>SVD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Britain, Scandinavia</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>France, Belgium, Italy</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Spain, Portugal</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Remaining Countries</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

*Porcine trypsin is possible source.
**Fetal bovine serum is possible source.

### Table 5. Approximate Costs 5/85 for Cell Line Testing Based on Country of Origin

<table>
<thead>
<tr>
<th>Country of Origin</th>
<th>Average Cost per Cell Line</th>
<th>Disease Agents tested for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>350 Bluetongue/EHD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Akabane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bovine Ephemeral Fever</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Hog Cholera</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>350 Bluetongue/EHD</td>
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<td></td>
<td>Akabane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bovine Ephemeral Fever</td>
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</tr>
<tr>
<td>Europe</td>
<td>2000 Foot-and-mouth Disease</td>
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<td></td>
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<td>South America</td>
<td>? Foot-and-mouth Disease</td>
<td></td>
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<tr>
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Table 6. Summary of Cell Lines Tested (10/1/84 to 5/31/85): Origin, Type, and Destination

<table>
<thead>
<tr>
<th>Origin</th>
<th>Type</th>
<th>Applied</th>
<th>Completed</th>
<th>Private</th>
<th>Destination</th>
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<tr>
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<td>Mouse</td>
<td>12</td>
<td>6</td>
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<td></td>
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<td>9</td>
<td>3</td>
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<td>Mouse</td>
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<td>16</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>France</td>
<td>Mouse</td>
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<tr>
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<td>1</td>
<td>0</td>
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<td></td>
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<tr>
<td>W. Germany</td>
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<td>4</td>
<td>4</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
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<td>Mouse</td>
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<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
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<td>Mouse</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Netherlands</td>
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<td>0</td>
<td></td>
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<tr>
<td>Switzerland</td>
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<td>0</td>
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<td>20</td>
<td>32</td>
<td>19</td>
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APPENDIX 3

REPORT OF THE EMBRYO MOVEMENT SUBCOMMITTEE OF THE IMPORT-EXPORT COMMITTEE

Chairman: Mr. Shelby V. Timberlake, Jr., Pelham Manor, NY

Dr. Steven R. Bolin, IA; Mr. Clint Booth, TX; Dr. Richard A. Bowen, CO; Mr. Dan B. Childs, FL; Dr. John A. Cobb, GA; Mr. Howard Hansen, VT; Dr. W. C. D. Hare, Canada; Dr. Duane C. Kraemer, TX; Dr. D. W. Luchsinger, NY; Mr. Joe Massey, TX; Dr. Charles A. Mebus, NY; Dr. T. D. Rich, MO; Dr. George O. Winegar, MD; Dr. Joseph M. Wright, TX.

The meeting of our committee was called to order at 10:00 a.m. on October 29, 1985, in the Westminster Room of the Marc Plaza Hotel, Milwaukee, Wisconsin.

A total of thirty-three attendees and members of the USAHA were present to discuss the movement of embryos in International Trade.

The chairman reported that Drs. Carmichael, Hare, Massey, Wright, Mr. Hansen and he had attended the IETS meeting in Montreal in January, 1985 and the recent meeting of the AETA in Louisville on October 23, 1985.

Resolution 2, Part I, which was passed at the 1984 USAHA conference asking the Secretary of Agriculture to urge his counterparts to consider
delaying issuance of rigid regulations until additional research data became available, was reviewed. There followed reports made by Drs. Waters, Stringfellow, and Carmichael describing the work done prior to the May 1985 O.I.E. meeting, the progress made by the IETS Task Force at a meeting with the Zoo Sanitary Code Committee on May 21, 1985, and the request by O.I.E. for IETS to produce a reference manual for the movement of embryos in international trade. It was further reported that Dr. Stringfellow has been invited to attend a technical session of the “Norms” committee of O.I.E. on December 9, 1985 along with Drs. Mapleton, Singh, VanderMaaten, Mahon, and Thibier. In view of our resolution stated above the meeting in May was considered an overwhelming success. Dr. W. C. D. Hare did a superb job for our industry at these meetings. The outlook for continued success at the December, 1985 meeting is bright.

Resolution 3 passed at our Ft. Worth meeting urging the Secretary of Agriculture to ensure support and foster research on disease transmission by embryo transfer and to form a work group of research, industry and USDA personnel, was restated and the chairman reported that the USDA had extended an invitation to AETA to meet with them in the very near future to determine research and industry priorities.

Dr. David Herrick of USDA/APHIS reported on the just published new rules for the international movement of embryos and the wish of his department to make these rules flexible enough to accommodate new advances in embryo technology to speed up the safe flow of embryos in international trade.

Dr. George Winegar of USDA, APHIS, then discussed the current export and import problems facing embryo movements in international trade and a constructive question and answer period followed.

Resolution 2.2 urging the USDA to sponsor a symposium of research leaders and regulatory officials and industry to exchange information on current research data and its implications was reviewed. The chairman reported that efforts had been made since 1984 to combine such a conference with I.E.T.S. and O.I.E. conventions but the schedules were not able to accommodate. A committee of Drs. Bolin, Carmichael, Waters and Messrs. Booth, Hansen and Timberlake was formed to amend resolution 2.2 (as attached), and have Mr. Booth approach Dr. John Atwell for assistance in arranging a symposium to be scheduled for June or July, 1986.

The meeting was adjourned at 11:45 a.m.

APPENDIX 4

COMMITTEE ON IMPORT-EXPORT — Export Subcommittee

Chairman: Dr. Harold Waters, Arlington, VA

Mr. William A. Bailey, MD; Mr. C. T. Barns, Jr., VA; Dr. J. L. Blair, VA; Mr. Clint Booth, ExOfficio Mem., TX; Frank Harding, IL; Mrs. Michele C. Howard, CA; Mr. M. E. Mix, VT; Mr. M. J. Nolan, DC; Dr. T. D. Rich,
The committee meeting was convened at 1:30 p.m., October 29, 1985, in the Chagall Room of the Marc Plaza Hotel, Milwaukee, Wisconsin; 18 committee members and observers were present. The following agenda items were discussed:

1. Diagnostic Laboratories — the National Veterinary Services Laboratory, Ames, IA now serves as a reference laboratory. All routine testing is now performed at approved laboratories. Exporters should contact the Veterinary Services (VS) office in the state of origin of the animals to obtain information on approved laboratories.

2. Accredited Veterinarians — health certification of animals for exportation must be done by accredited veterinarians. There are not enough full time government employed veterinarians to provide this origin inspection, testing, and certification. VS is increasing the enforcement of regulations governing accredited veterinarians. This has resulted in increased publicity regarding accredited veterinarians. It was the view of the committee that to foster world-wide confidence in the accredited veterinarian such publicity should reflect the positive results of the accreditation system as well as the results of such enforcement action.

3. Export Task Force Committee — The progress of this USDA appointed committee action was reviewed. The Export Subcommittee will review their report when completed for possible USAHA recommendations.

4. Export Facilities — availability and adequacy of export inspection facilities were reviewed. It was noted that as specially designated export inspection facilities are increasingly approved and used, this diversion from approved facilities at designated ports of embarkation has resulted in deterioration of these permanent facilities. It was recommended that permanent port facilities be reserved for back-up use at the port if animals previously certified at a remote temporary facility should be delayed at the port of embarkation.

5. Export Animal Health Program — Dr. George Winegar, gave a report on the Export Animal Health program. A copy of this report is included with the VS report to the Import-Export Committee. Dr. Winegar also introduced the International Regulations Retrieval System (IRRS). This system for accessing on computer the import requirements of specific countries has recently gone on line within VS. Plans are being made for other people to access the system through a subscription network. The Export Subcommittee commends VS for this progressive step.

The meeting was adjourned at 3:15 p.m.

Respectfully submitted,

Harold A. Waters, Chairman
INTRODUCTION

Falsifying U.S. Department of Agriculture animal disease tests records by using blood from a single donor to represent that from a number of animals is a serious violation of Title 18, U.S. Code, Sections 1001 and 2.

Although such violations of the Federal Code have been going on almost since the start of the Brucellosis Eradication Program in cattle, recent evidence would suggest that the incidence of such violations may be greater than previously suspected.

One of the most sinister features of such crimes is that they can lead to the spread of disease by the introduction of infected animals into areas that have been certified as disease free — to wit, the recent case involving Ernest Mendel, a New York cattle dealer (re: the 3/12/84 news release of the USDA’s News Division).

Although the aforementioned news release stated that “expert witnesses testified at the trial that blood samples used in the (brucellosis) tests were taken from two healthy bulls — not from Mendel’s infected cows,” nothing was mentioned in the news release concerning the nature of the incriminating evidence. In a nutshell, the evidence was obtained by performing hemolytic blood typing tests on red blood cells freed from the clots and showing that there were only two different blood types represented in the multiple blood samples submitted by Mr. Mendel to the Albany laboratory for brucellosis tests. Eventually blood samples were obtained from the two bulls en route to slaughter, and it was found that the two different blood types of the bulls matched perfectly with the two different blood types in the aforementioned blood samples. When blood samples were obtained from a number of the cows that had been falsely represented, it was found, as expected, that each was of a different blood type, a type clearly distinct from the types of the two bulls. The evidence, which was most conclusive, was not contested by the defense.

Let us turn now to a brief discussion of the nature of the blood typing tests and their efficacy in identifying individual animals.

The Serological Tests

The membrane of the red blood cell is literally peppered with a great variety of antigenic determinants, commonly referred to as blood factors. Some of the blood factors are fixed genetically, i.e., they occur on the RBCs of all members of the species under study. Others segregate genetically,
i.e., they occur on the RBCs of some but not all members of the species under study. It is these segregating determinants which form the blood groups proper and define individual differences.

In cattle some 80 of these alloantigenic or segregating determinants have been brought to light and they have been assigned to one or another of 11 genetically independent systems of blood groups. Some of these systems involve only one or a few blood group factors whereas others are very complex. For example, the B system which involves slightly over one-half of all the cattle blood factors, is by far the most complex blood group system identified to date in any species.

Other species of domestic animals (e.g., pigs, sheep, goats and horses) have considerable numbers of RBC alloantigenic determinants but none except pigs comes close to having the number identified in cattle. Nevertheless, those numbers, ranging from about 30 in horses to 65 in pigs are (re Stormont, 1982 and Bell, 1983 more than adequate for purposes of identifying individual animals.

The vast majority of these blood factors on animal RBCs have been brought to light by the production and use of alloimmune (formerly isoimmune) antisera as the source of blood typing antibodies or reagents. To date none of these reagents has become commercially available thereby limiting the number of blood typing laboratories which are capable of performing these tests. In a 1982 report the author stated that there were some 30 laboratories worldwide which provide cattle blood typing services and 26 which provide horse blood typing services. These and other statistics on animal blood typing may be found in the two recent reviews already cited.

Sometimes only serum from the clotted blood samples is available for typing and this brings us to the use of gel electrophoresis as a means of identifying serum samples from different individuals.

There is in most mammalian species a number of serum proteins which occur in more than one electrophoretic form. And each of these electrophoretic forms of a given protein, as serum albumin, is coded for by one of a series of allelic genes. For example, in horses there are two common electrophoretic forms of serum albumin encoded by a pair of allelic genes, Al^A and Al^B and these alleles combine to form three albumin types designated A, AB and B. In horses there are also seven relatively common allelic genes for different electrophoretic forms of the iron-binding serum protein known as transferrin. These seven alleles combine in pairs to produce 28 transferrin types. Because each of the albumin types is inherited independently of the 28 transferrin types there are 84 possible blood types when considering these two genetic systems alone. Of course, these numbers multiply when considering additional systems (re: Stormont, 1979) but the expense involved in running all the different electrophoretic tests is prohibitive. Therefore for the purpose of positive animal identification we here in Stormont Labs are making considerable use of the method of polyacrylamide gel electrophoresis (PAGE) described by
Gahne et al. (1977). On a single PAGE gel we can, for example, resolve a number of serum protein types in such species as cattle, horses, pigs and sheep.

**Efficacy of the Blood Typing Tests**

The **serologic tests.** We routinely employ 60 different blood typing reagents in the cattle blood typing tests and these reagents are theoretically capable of distinguishing trillions of different blood types. Thus the probability of drawing two blood samples from a single breeding population that are exactly alike in blood types is less than one in a million.

In horses where the number of alloantigenic determinants on RBCs is far less than in cattle it is not too uncommon to find that two animals from the same breeding population do, by chance, have like blood groups in all systems.

However, these odds can be increased considerably by performing a variety of gel electrophoretic tests. For example the odds of randomly drawing two Thoroughbreds that have identical types in all the systems commonly tested for are about one in 25,000 whereas the odds when considering Quarter Horses are about one in 250,000 (Stormont, 1979). Thus it is seen that the tests are extremely powerful in distinguishing between individual animals.

The **electrophoretic tests.** When serum samples only are available for the tests we are of course limited to the number of blood types which can be defined. In horses this is no problem because of the extensive allelism involving such serum proteins as the protease inhibitors (Pi), formerly referred to as prealbumins (Pr), and the esterases and transferrins. In cattle the allelic diversity, at least for the more common alleles is much less. For example, when running cattle serum samples in PAGE gels we commonly encounter only 3 albumin (Al) types, 3 group-specific component (Gc) types, now known as vitamin D binding protein, 10 transferrin (Tf) types and 3 post-transferrin (PTf-2) types. The total number of types is $(3 \times 3 \times 10 \times 3)$ or 270 types. In certain breeds there is virtually no genetic variation in Al types. For example, virtually all Holsteins are of Al type A thus making this system almost useless in identifying individual Holsteins and thereby reducing the effective variation to just 90 types in this breed. Therefore it is not unusual when running PAGE gels on 10 different Holsteins to find as many as 3 or 4 that are of the same type.

In horses, because of the greater allelic diversity and an additional system, namely, serum esterase (Es) which can be defined in PAGE gels, the number of different types is 9,072. Thus, the odds of drawing two horses from the same breeding population that have the same type on PAGE gels are considerably less than cattle.

In closing this account, I know you will all be interested in the fee basis. We can only speak for ourselves. For the standard cattle blood typing tests, which involves only the tests for the RBC alloantigenic determinants, the current fee is $25.00 per blood sample. For the system of electrophoretic
markers our current fee is $5.00 per system.

In horses where the standard tests include not only the RBC antigenic markers but also a variety of serum proteins, the current fee is $30.00 per blood sample. As in cattle, the fee for any system of electrophoretic markers is $5.00.

When serum samples alone are available we routinely perform tests only for those markers which can be defined on the PAGE gels and the fee is $20.00 per sample irrespective of species.

However, on a contract basis, with a guarantee of a specified number of blood samples to be tested annually, these fees could be considerably reduced.

REFERENCES
Recent Progress in the Development of Animal Electronic Identification Systems

Sidney L. Spahr and Hoyle B. Puckett

Development of commercial systems for electronic identification (ID) of livestock is the major technological breakthrough that is allowing the involvement of livestock automation. The first system appeared commercially in the U.S. about 1979. Since that time the newer units have become smaller, more reliable, cheaper, and have a greater capacity for animals on a single system. Some of the commercial systems now available have a capacity of 10 billion different numbers, and some of them may be programmed to have sequential numbers for a specific installation after they leave the factory.

Industrial development of electronic identification is continuing, and several companies are working toward specific applications in livestock production. Some of the technical features of electronic identification (ID) systems are shown in Table 1. Most of the ID units worn by the cow are powered by radio frequency (rf) energy emitted from a stationary transmitter. These units are typically located at a feed dispensing stall, in a milking parlor, or at some other location where animals will be confined. When the ID unit worn by the animal becomes powered up, it transmits an electronically coded unique signal which is received by an antenna and then decoded and matched electronically with the herd name or number of the animal wearing the specific ID tag.

In our research we have studied a number of features which make some systems more suitable than others for specific applications. For example, some applications require that the ID unit be attached permanently to the animal. Implanting is not feasible with most of the systems because the ID units are too large. In addition the range of interrogation for most systems is so small, especially when ID units are implanted, that many potential applications are impracticable.

Major improvements in the life of batteries have led some developers to design their ID units to be powered with batteries to increase the range of interrogation. This approach is useful if the user desires to attach a physiologic sensor to an electronic ID unit. Examples of such units under development for livestock are temperature sensors, activity tags, and tissue composition sensors.

Recent Developments

Several new systems utilizing new technology have emerged commercially during the past year. Allflex International, Ltd., a company well known for their worldwide sales of animal ear tags, is the marketer for the first commercial system which uses surface wave technology as their

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The authors are Professor of Animal Sciences, University of Illinois at Urbana-Champaign and Research Leader (Retired), US Department of Agriculture-ARS-NCR.
method for identifying animals. The ID unit for this system consists of a small man-made crystal and antenna embedded in an ear tag. The ID number is factory programmed on the crystal with more than 250 million codes available. It operates at only .001 watt power and the ID units are light enough to be supported easily in the ear of cattle, sheep, or swine. Company representatives estimate the commercial availability of this system by mid-1986, with the first application being the automatic weighing and recording of livestock weights. This system has sufficient range of interrogation to allow animals to be identified as they walk through an archway, a feature which allows many applications.

Dairy Equipment Company, manufacturers of Boumatic equipment, also has developed a new ID unit with sufficient range for archway identification. Their unit will be used initially to identify cows as they enter a milking parlor. The identity of each cow will be linked to an electronic milk measuring meter and recording processor to provide an automatic system for recording milk weights on every cow at every milking. Their unit is designed to be worn around the neck and attached to a chain or strap. Company representatives expect this system will be available commercially no later than the first quarter of 1986.

The "Eureka Intelligent Tag", manufactured by the Eureka Division of Senelco, Ltd, in Slough, England, is an example of an electronic ID tag developed for the security market which is finding a place in livestock production. This tag measures 31mm x 29mm x 9mm, one of the smallest ID units available. It is battery powered and is attached to cattle as part of the animal’s eartag. Fearing International, Ltd. of England makes an ear tag specially designed to hold the electronic ID unit.

We installed the Eureka system in 8 stalls of a 16-stall milking parlor on the University of Illinois dairy research farm during March 1985. The system is interfaced with a computer which controls the order and rate for polling at each stall, and integrates the identification data with milk yields and milk conductivity data being collected in real time at each stall. Our experience indicates that the range of interrogation is 18 to 24 inches in the parlor with the antennae supplied by the company. We have increased the range of interrogation by about 50% in the laboratory by using a larger antenna. Batteries have completely discharged on a few units, but the system continues to function well in our facility after about 6 months continuous use with every-milking identification of about 60 cows.

The first animal ID unit sold in quantity for livestock ID and designed specifically for implantation was introduced in late 1984 by Mix Mill, a division of Blount Industries, in Bluffton, Indiana. The unit is made by Farm Technology, Inc. as part of their developing line of automatic feeding and milk measuring equipment. The units also may be mounted around the neck in a manner similar to the ID units sold for automatic feed dispensing by other companies. This unit has undergone sufficient miniaturization and packaging to allow implanting, but only has a range for interrogation of about 6 inches.
The smallest electronic animal ID unit is the miniaturized model manufactured by Identification Devices, Inc. (IDI) for implantation. This unit is small enough to be utilized for identifying salmon during migration for spawning. However, its application for livestock ID is limited by its short range of interrogation (2 in.).

After suitable electronic ID systems are developed for livestock an additional task of interfacing the ID system for specific applications is necessary. This is no small task for most applications. It often requires the development of specialized instruments or equipment, and almost always requires that a professional programmer write a special purpose data collection and analysis program. An example of the problems which must be solved is found in our program for automatic collection of data in the milking parlor, Figure 1 (Puckett et al. 1985, Spahr et al. 1985).

One of the first problems is how to have readers located at multiple locations, but to have only one data communication line coming into the computer which stores and analyzes the data. This problem usually is solved satisfactorily by multiplexing, but a multiplexor is an extra piece of equipment that usually adds several hundred dollars to the single-reader demonstration units shown by electronic ID vendors at trade shows. If the ID unit is to be linked to a measurement sensor, for example a milk meter, the data collection program must have some automatic way to separate the measurement of one animal from the measurements of the next one. In the milking parlor we use pressure sensors on the entrance and exit gates and we monitor on the vacuum switch for the milking machine to open and close files for each animal. Each application of electronic ID is different, but problems similar to these must be solved in order to incorporate electronic animal ID into an automatic data collection system regardless of whether it is installed at a livestock sales facility, at a slaughterhouse, or on the farm.

Acknowledgement

This paper is based on a study conducted under project No. 35-0365 of the Agricultural Experiment Station, College of Agriculture, University of Illinois, entitled Electronic Identification for Dairy Automation. It was supported in part by a grant from the US-Israel Binational Agricultural Research and Development Board as part of Project No. US-439-82, Automation and Electronics for Dairy Herd Management.

REFERENCES

Table I. Major Electronic Animal Identification Systems

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<tr>
<th>Manufacturer</th>
<th>Address</th>
<th>Battery or Passive</th>
<th>Animal Attachment</th>
<th>Range of Interrogation</th>
<th>Comments</th>
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<tr>
<td>All Flex</td>
<td>Walnut Creek, CA</td>
<td>Passive</td>
<td>Ear</td>
<td>3 ft +</td>
<td>Suitable for archway ID</td>
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<tr>
<td>BI (Boumatic Harvestore, Zero)</td>
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<td>Passive</td>
<td>Neck</td>
<td>6 in</td>
<td>Surface acoustical wave</td>
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<td>Boumatic</td>
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<td>2-3 ft</td>
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<td>Neck</td>
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<td>Neck</td>
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<td>Switched on by magnet</td>
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<td>12 in</td>
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<td>Ear</td>
<td>18-24 in</td>
<td>Powered up by Rf</td>
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<td>Farm Technology (Mix Mill)</td>
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<td>Implant or Neck</td>
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<td>UIS (Pinpointer)</td>
<td>Cookville, TN</td>
<td>Passive</td>
<td>Ear</td>
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</table>
Figure 1. Automatic data acquisition and analysis system for dairy farms.
REPORT OF THE COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: R. E. Nelson, Brattleboro, VT
Vice Chairman: Dewey Bond, Washington, DC

J. B. Ashcraft, CO; P. E. Bradshaw, IL; D. R. Bridgewater, CO; D. E. Casey, MO; H. F. Embry, IL; G. B. Estes, VA; R. Gadd, SD; W. Gallagher, SD; H. E. Goldstein, OH; T. Haas, KY; J. N. Huff, CO; G. M. Jones, NM; R. Jones, SD; D. Likes, KS; M. Main, SD; G. Mulder, IA; N. F. Powers, Jr., NY; E. C. Roukema, VA; R. Schnell, ND; K. W. Scritchlow, IL; G. L. Seawright, NM; R. S. Sechrist, OH; G. R. Snyder, VA; W. E. Stemler, IL; F. E. Sterner, CO; J. E. Thomas, AR; C. R. Watson, MD.

The Livestock Identification Committee met Wednesday, October 30, 1985, with 62 attending, including 19 members of the committee.

Dr. Cecil Watson, USDA-APHIS-VS, reviewed the results of studying the feasibility of incorporating bar codes on backtags to allow the electronic input of the backtag numbers into the Brucellosis Information System. He reported nearly a 90% read rate in the study using laminated and non-laminated backtags and lo-density and high-density bar codes using an infrared wand. This success prompted implementing a pilot study in five states during fiscal year 1986.

Dr. Robert Whiting, USDA-APHIS-VS, updated the committee on revised wording of CFR parts 71.18 and 78.30. With this wording incorporating the term interstate commerce instead of interstate movement makes it illegal to remove ear tags and/or backtags between the point of origin and the point of final destination. Final rule-making is due for publication within the next few weeks.

Mr. Wayne Scritchlow spoke on behalf of Dr. Dewey Bond and reported on the AMI position requesting mandatory identification of all swine arriving at market that moved interstate as USDA is authorized to do if it is not authorized to require identification of all swine arriving at all markets irrespective of location.

Dr. G. R. Snyder of USDA-FSIS reported that FSIS was not yet in a position to offer any revision in its position but the report of the National Academy of Science was prompting reconsideration. Separately it can be noted that this report emphasized USDA’s responsibility to protect the food supply and given advances in science and technology, deficiencies should be corrected.

Dr. David Meisinger, Director of Research, National Pork Producers Council, commented on the pork producers perspective on identification. He pointed out that at a recent meeting of the NPPC Swine Identification Task Force, it acted to support mandatory identification of all slaughter hogs back to the last farm of ownership with encouragement for sharing carcass quality and health information with the producers. Dr. Meisinger went on to identify all of the benefits that could accrue from feedback
information from the packer. He described the cooperative project funded by USDA-FSIS at Michigan State University to do slaughter checks with information released back to the producers. He then discussed the possibilities for a national numbering system for animals that could interface with identification of gambrels that would be electronically read, thus gaining the capacity to trace the animal and its carcass from the farm of origin through the slaughter plant. With policy to be developed at the annual meeting of NPPC, he predicted movement toward a mandatory swine identification program.

Mr. Bill Gallagher, former Chairman of the National Cattleman's Association Herd Health Committee, commented on the cattle producers' perspective on identification. He affirmed the use of Hot iron brands as the most effective ID system in the world, recognized the problem of the swine industry with its sulfa residue problem and emphasized the NCA opposition to government mandated identification systems because they are costly and unnecessary.

Dr. Austin Knosby, Osceola, Iowa, consultant to Austin Enterprise Systems, Inc., reviewed several identification systems he had tried. He then described a system of attaching a bar coded tag to the inside of the ear of a hog with a Dennison T. Tagger. He explained successful use with veal calves as well.

Mr. David Bowers, of Sentel Technologies, Inc., La Mesa, California, described bar code applications to identification within a herd, particularly a dairy herd. This included identification of animals with tags encircling the tail, along with identifying milk samples and reading milk measuring devices — all with laser scanners feeding into microprocessors. The bar code completely encircled the tail and any round sample containers which were also color coded.

Mr. Richard Robinson, of Agriculture Canada, described the Red Meat Products Division's plan to implement a field trial whereby 1,000 beef animals will be identified by cooperating producers with an electronic device that has passed the bench test. These cattle will be marketed via normal marketing procedures. Field electronic identification will be integrated with slaughter facility electronic identification, all to be followed by post-trial evaluations. Mr. Robinson also commented on the separate project in the Province of Saskatchewan in which 30 steers and 30 first calf heifers were implanted with I.D.I. devices in May 1985. The steers will be electronically monitored at fall weaning and spring weighing, with heifers electronically monitored fall, winter and spring for three to five years.

Dr. S. L. Spahr, of the University of Illinois, described advances in development and application of 12 different electronic identification devices. While listing and showing externally attached neck mounted units becoming common in the dairy industry to automate feeding, he discussed in more detail those attached to the ear and being marketed for identification purposes, one of which is field programmable and all of which have
the potential for expanded cattle management functions. He cited applications to swine in England.

He also commented on the most recently developed implantable device being marketed by Farm Technology, of Bozeman, Montana, which was battery powered with six inches of range.

The manner in which these devices were tried and applied in the University of Illinois herd was described, all of which will be covered in a separate paper.
TRICHINOSIS SAFE PORK AND THE ROLE OF THE ELISA AND "STOMACHER" TEST

Diane G. Oliver, M.D., F.C.A.P., Rita Hanbury, B.S., C. Donald Van Houweling, D.V.M.

Delegates to the National Pork Producers Council’s Annual Meeting in 1982 suggested formation of a national task force to study the issue of trichinosis and to recommend methods for its elimination. At the first meeting of the task force, it was decided that total eradication of trichinosis from the U.S. was probably not possible, but that delivery of a trichina-safe pork product could be achieved.

The group, calling themselves the Trichina-Safe Pork Task Force, is made up of pork producers, pork packers, industry representatives, several government agencies, university people and other interested individuals. They have set 1987 as their goal to achieve a 100 percent retail pork supply safe from the threat of trichinosis.

Human trichinosis is not a very prevalent human health problem in that only one hundred or less human cases are reported each year in the U.S. The motivation of NPPC in eliminating the problem of trichinosis relates to consumer attitudes and perceptions. Consumers generally know that one must cook pork until it is well done to be trichina safe. A problem arises because most consumers overcook the product and then complain because it is dry and tough. NPPC is convinced that demand for pork would be significantly enhanced with assurance of a product safe from trichinosis.

Detection of trichinosis has been used in Western Europe since the early 1900's. The detection methods presently of choice are the pooled digestion technique and the ELISA test. The control methods are cooking, curing, freezing, and irradiation.

There are already existing regulations for processing and for freezing. Irradiation is a new technology that is just now receiving much attention.

A major economic feasibility study conducted about two years ago anticipated a two percent increase in domestic demand and a ½ increase in exports brought about by certifying pork as trichina safe. That study projects an almost ½ billion dollar annual return to pork producers, pork packers, and retailers from trichina-safe pork. The preliminary results of an internal study being currently conducted by USDA demonstrates that these estimates may be conservative.

Market research indicates that consumers may be willing to pay a few more cents a pound to be assured that fresh pork is guaranteed trichina safe. In consideration of the fact that there are 140-160 pounds of salable pork cuts in the average carcass, this extra guarantee could provide a bonus of a few dollars per hog or carcass to the industry. This program is truly one of adding value.

Implementation of a “trichina free pork” program involves the producer, packer, consumer and government. One approach being discussed involves
labeling of trichina-safe pork to achieve this value-added scenario. However, many are concerned that such labeling would imply that unlabeled pork is infected with the causative organism. Another approach being discussed would have testing as an integral part of all meat inspection, thereby assuring all pork trichina safe. With this approach, a national consumer education program could emphasize the new safe pork for maximum appeal from a taste, tenderness and juiciness perspective.

**STOMACHER**

The State of Illinois has been a leader in trichina eradication. Since its inception, the Illinois Trichinosis Control Program has been structured to utilize the pooled sample digestion technique as the primary laboratory procedure. In the early 1970’s, the Zimmerman 18 hour incubation system was used. Today, the Stomacher method is used for detection of *Trichinella spiralis* larvae in tissue. In the late 1960’s a national Trichinosis Eradication Program was designed. Unfortunately, that plan was not placed into operation. However, it did initiate a long range goal in Illinois to establish a trichinosis control program.

A Zimmerman trichina incubator was loaned to the Illinois Department of Agriculture in 1970 which initiated a Trichinosis Survey. Identified swine diaphragm samples were collected from state licensed swine slaughter establishments. From January 1971 to December 1974, 50180 swine samples were collected and analyzed. Infected premises were found in three geographic locations, Knox County in west central Illinois, the east St. Louis area, and east central Illinois close to the Indiana border. When it became evident there was no interest in elimination of the infected herds and the national program plans were discontinued, the Illinois program was halted.

The action of the National Pork Producers Council along with a positive response from the Illinois Pork Producers Association brought about reactivation of the Illinois Trichinosis Program. It was kicked off with a planning session on January 13, 1984.

Through the efforts of the National Pork Producers Council, two Stomachers were loaned to the Illinois Department of Agriculture. As these units had primarily been used for trichina control efforts in Europe, it was necessary to develop a procedure maximizing the European method while adhering to U.S.D.A. policy. Since the inception of this endeavor in March 1984, the procedure has been refined and efficiency substantially improved. Currently, analysis of twenty (20) samples or one pool can be accomplished in one and one-half (1 ½) hours.

The pooled digestion technique involves combining up to twenty tissue samples to form a “pooled” sample. If a positive pool is found, tissue from each individual sample is tested to determine which animal(s) were infected.

The principle behind this technique is the homogenization or breakdown of tissue in a hydrochloric acid and pepsin solution. The “Stomacher”
provides the mixing action and is designed to simulate the peristaltic motion of the stomach. After homogenization is complete, the solution is filtered and larvae (if present), are sedimented, retrieved and viewed under a dissecting microscope.

The Stomacher method has been used in three laboratory facilities in Illinois.

The first was the placement of a Stomacher based laboratory in a small swine slaughter establishment. This was done to test the practical and economic feasibility of the use of the Stomacher method for trichina detection on an in-plant basis. Testing was performed and completed the same day of slaughter so that theoretically carcass certification and dispatch could be accomplished as soon as the test results were available. This laboratory was in operation from March 1984 to March 1985.

The second facility is a diagnostic laboratory located in Springfield, Illinois and has been utilized to reinstitute the Illinois State Survey. This laboratory has been structured to maximize efficiency and is staffed by one full-time laboratory technician using two Stomacher units. This laboratory was established in March of 1984.

The third facility is located in the Animal Disease Laboratory located in Centralia, Illinois. It is also utilized in the Illinois State Survey analysis. This laboratory began testing in June 1985 on a part-time basis. It serves as a model for a small scale diagnostic laboratory.

37,977 swine diaphragm samples have been analyzed in these laboratories using the pooled digestion technique.

To date, no new foci of trichina infection have been located in Illinois. The East St. Louis and eastern Illinois farms remain infected. The Knox County foci has been eliminated partially due to discontinuance of swine production on the infected premises. Swine from the Knox County area have been sampled extensively since March 1984 with negative results.

With financial support from the National Pork Producers Council and the Illinois Pork Producers Association along with cooperation of the herd owner, a research project designed by Dr. K. Darwin Murrell, Animal Research Services, U.S.D.A., was conducted on the eastern Illinois infected farm. This project established for the first time under controlled research that cannibalism can be a major factor in maintaining trichinosis infection in a swine herd.

The results of the 1970-74 survey were not correlated with the farms of origin when the test was negative. The present state survey has been computerized and a recent list of Illinois pork producers has been obtained which will permit the correlation of positive and negative samples to the farms of origin. As the present survey is expanded to encompass the entire state, hopefully it will be possible to determine within a reasonable estimate the percentage of Illinois swine production units that have been surveyed. Inquiries of major Illinois pork producers indicate that the majority have swine slaughtered in state inspected establishments, and therefore, not subject to the trichina survey. Hopefully, the swine slaugh-
tered for the home freezer will result in most producers being included in the survey.

Through sponsorship of the Illinois Pork Producers Association, the Illinois Trichinosis Control Act was written and enacted into law with an effective date of January 1, 1986. This act provides the basic authority for a disease control program. The Illinois Department of Agriculture is authorized to quarantine trichina infected herds, require testing of swine suspected of being infected with trichinosis, and if state funds are available, indemnify owners of swine destroyed due to trichina infection. The Illinois General Assembly appropriated $120,000 to indemnify owners for destroyed trichinosis infected swine. Implementation of the Illinois Trichinosis Control Act is scheduled for January 1986.

As soon as federal meat inspection has certified the two Illinois Trichinosis diagnostic laboratories, a voluntary program will be established for state inspected swine slaughter establishments. Diaphragm samples will be collected at approved establishments from all swine at the time of slaughter and forwarded to one of the certified laboratories and examined for presence of trichina. If negative, the carcasses and meat products will be certified trichina safe.

Future activities in the Illinois program will include continued surveillance for trichina infected herds and cooperation with the national plan for trichina safe pork for all United States citizens.

ELISA

The ELISA test (enzyme linked immunosorbent assay) for swine trichinosis has received considerable attention recently because of the sensitivity and potential for automation. Appreciable work has been done by the USDA and universities in the development of a test suitable for use by the pork industry. This summer the National Pork Producers Council and the North Carolina Pork Producers funded a project to study the feasibility of using the ELISA test in a commercial high speed pork slaughter house in North Carolina.

Procedures and Methods

Blood samples were collected on 3005 hogs at the time of slaughter over a period of three weeks. Each collection tube was prelabeled with machine readable numbers and the animal tattooed simultaneously with the same number for later identification. A sample of diaphragm was taken from each pig for analysis by the stomacher technique. This tissue was divided and sent both to the State of Illinois and to the USDA at Beltsville. Duplicate blind analyses were done. The blood was taken to the laboratory located at the packing house and analyzed by ELISA. Each sample was performed in duplicate and controls were run on each plate.

RESULTS

All ELISA results were available within two hours from the time the samples were received in the laboratory. Day-to-day precision of the assay
was excellent, as was sample-to-sample reproducibility. Specificity and sensitivity of the procedure in the field was comparable to that achieved in a research laboratory setting. The system used in the field trial was capable of handling up to 3000 samples per day at a total cost of approximately 1 cent per pound of marketable product, including collection and overhead. This system can be improved with additional automated equipment to handle kill line speeds of 1000 hogs per hour.

CONCLUSION

It is apparent from the field study that the ELISA system can be automated to be used in large scale packing plants for the certification of “trichina-free” pork. In addition, the stomacher technique is useful for testing in smaller plants. Together these two diagnostic methods provide the means to screen hogs both on the farm and in the slaughterhouse in order to make the goal of trichina-free pork finally a reality in the United States. The State of Illinois has set the pace, market studies have shown that the consumer will pay more for a trichina-safe product, and methods are presently available to economically test slaughtered pigs for this parasite. The decline in pork consumption can be reversed with this safer, more palatable product.
REPORT OF THE COMMITTEE ON PARASITIC DISEASES AND PARASITICIDES

Chairman: John F. Hudelson, Denver, CO
Vice Chairman: John H. Gray, Aurora, CO

This committee met on Monday, October 28, 1985 at the Marc Plaza Hotel, Milwaukee, Wisconsin.

The meeting was called to order by the chairman. Approximately 25 persons were in attendance, 13 of which were committee members.

Dr. M. G. Scroggs, Manager, Technical Service, Southwest Region, MSD AGVET, Merck and Company, Incorporated, presented an update on the use of IVOMEC in the United States since approval in 1984. Information on Curatrem (Clorsulon), a new flukicide, was also presented.

Dr. R. O. Drummond, ARS, USDA, Kerrville, Texas, reported that true resistance of horn flies to pyrethroids in insecticidal eartags has been documented. Studies are looking at genetics and how resistance is maintained by the flies. He suggested producers should not use insecticidal ear tags where resistance has occurred, instead use other means of fly control; such as dust bags, sprays, and oilers with chemicals other than pyrethroids.

Dr. Drummond also discussed the resistance of cattle fever ticks in Mexico to organophosphates. He stated that Mexican tick control program had collapsed. He mentioned that amitraz has shown no resistance by ticks in Australia for 14 years.

Dr. Drummond stated that a bacterium, Flavobacterium sp. had been used to degrade coumaphos dip when disposed. Twenty-four hours after the inoculum has been added to the dip, an ozone generator is used to provide ozonation which causes fragmentation of the benzene ring and destruction of the micro-organisms.

Dr. Chester Gipson, USDA, APHIS, VS, discussed the tick situation in Puerto Rico. He also stated that the reported scabies cases for fiscal year 1985 was 35 compared to 75 in 1984. He stated that heartwater in cattle in the Caribbean was being monitored. A diagnostic test is under development while the distribution of the disease is plotted.

A proposed resolution relating to heartwater was presented to the committee as proposed and supported by NCA. This resolution was passed.
by the committee and is referred to the resolutions committee to be presented to the USAHA for passage.

Dr. Gary Zimmerman, College of Veterinary Medicine, Oregon State University, Corvalus, Oregon, presented information on *Nematodirus battus*, a nematode parasite of sheep. This parasite is reported to be a foreign animal disease. Dr. Zimmerman has found this parasite in sheep in Oregon five times during 1985. Concern was expressed that animals are being imported into the United States with disregard for presence of internal parasites. He suggested that local, state and federal authorities as well as livestock groups investigate the distribution of this parasite. A resolution regarding this parasite was tabled.

Dr. Darwin Murrell, USDA, ARS, presented information on an ELISA test for *Trichina* in pork. The test appears to be 96–97% accurate. The major expense of this test is the collection of the blood and identification of animals.

This committee adjourned after a very informative meeting.
NEWCASTLE DISEASE VIRUS INFECTION OF PIGEONS — IS IT A THREAT TO POULTRY?

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SUMMARY

In 1981, an outbreak of neurotropic Newcastle disease (ND) in European pigeons was reported. Between 1981 and 1984, the disease spread through Europe, Great Britain (GB), and into the United States (US). This strain of ND virus (V) was isolated from pigeons in seven northeastern US states in 1984. The only occurrence of Newcastle disease in domestic poultry due to this strain of NDV was in Great Britain in 1984 when 23 susceptible chicken flocks were affected due to feeding on contaminated feed. Experimentally, chickens placed in contact with infected pigeons or inoculated with the US virus by the intranasal route did not develop clinical disease, but did develop antibody against NDV. It appears that this strain of NDV poses a minimal threat to poultry; however, precautions should be taken to insure that infected pigeons do not come in contact with poultry.

INTRODUCTION

Before 1981, there were few reports that NDV caused clinical disease in pigeons. Also, pigeons have had a very limited role in the transmission of ND to poultry. Between 1981 and 1984, a strain of NDV that was pathogenic for pigeons appeared in Europe, and spread through GB, and into the US. The disease consisted of diarrhea followed by central nervous signs that included ataxia, tremors, wing and leg paralysis, and torticollis. A virus was isolated and was identified as paramyxovirus 1. NDV is the prototype strain for this group.

Erickson et al have reported on the experimental inoculation of pigeons with velogenic viscerotropic NDV.5 The clinical signs observed in pigeons inoculated by parenteral routes were similar to those reported in the recent outbreaks. A range of 17–83% of different groups of birds inoculated by the intranasal route developed clinical signs, but all developed antibody against NDV. The NDV vaccines that were available provided little protection against the disease.

Pigeon NDV infection in Europe and Great Britain

The disease spread through most of Europe in 1981 and 1982 and to GB in 1983.2,4 By 1983, most captive-raised pigeons in Europe had been vaccinated. There have been no reports of this virus infecting European poultry except in GB. This was probably due to the immunity level in the European poultry and pigeons.

In 1984, there was an outbreak of ND in poultry in GB due to a virus strain that was identical to the pigeon strain.3 Chickens on 19 of 23
premises were infected by feed contaminated with NDV-infected pigeon carcasses. None of the flocks had been vaccinated against NDV. The clinical signs observed were a drop in egg production (20/23), neurotropic disease (6/23), diarrhea (5/23), and increased mortality (5/23). No clinical disease was observed in 2 flocks.

Due to the outbreak, 820,000 chickens were slaughtered and the GB NDV eradication policy was modified to allow vaccination. There were several unique circumstances that caused this outbreak: large numbers of feral pigeons had become infected, the feed supply for poultry had been contaminated and NDV vaccination of poultry had been stopped in 1981 as part of an eradication program. Since 1984, pigeon and poultry have been vaccinated, and there have been no subsequent outbreaks.

**Pigeon NDV infection in the US**

In April 1984, clinical disease in pigeons identical to that observed in Europe and Great Britain was reported in New York, NY. A virus similar to that isolated in Europe and Great Britain was subsequently identified in specimens from 35 premises in 7 states submitted to the National Veterinary Services Laboratories (NVSL). The premises were located in New York, Vermont, New Jersey, Maryland, Pennsylvania, Delaware, and Virginia. Virus was isolated from specimens collected in April, May, June, November, and December of 1984. In 1985, isolations were made in March, April, May, June, July, and August. One of the US isolates was from a feral pigeon in New York City, NY, while the remaining 34 isolates were from captive pigeons. There has been no evidence of infection of US poultry by this virus.

**Pathogenicity of pigeon NDV for chickens**

Six- to eight-week-old chickens inoculated by the intranasal or cloacal route with pigeon NDV did not develop clinical disease. In studies conducted at the NVSL, 21 isolates that produced clinical disease in experimentally inoculated pigeons also produced lethal neurotropic disease in all one-day-old chickens inoculated by the intracerebral route. The average intracerebral pathogenicity index (ICPI) was 1.22; the range was 0.94 to 1.41. This is the same as the velogenic strains of NDV. The intravenous pathogenicity index of 10 isolates was determined by inoculating six-week-old chickens. Two of the isolates produced neurotropic clinical disease in half of the birds inoculated; however, some isolates produced no clinical disease. The average intravenous pathogenicity index was 0.40 which is the same as the lentogenic strains. The range was 0.00 to 1.30.

No clinical disease was observed in three chickens placed in direct contact with inoculated pigeons that later died from infection with the virus. All chickens developed antibody against NDV and the virus was reisolated from two of the three chickens. The mean death time in embryonating chicken eggs of all of the pigeon strains was greater than 90 hours. On this basis, the strains were classified as lentogenic NDV isolates.
Alexander *et al* reported that the ICPI of the GB isolate could be increased by back passage in chickens. However, no clinical disease was observed in chickens inoculated by the intramuscular route with virus that had been back-passaged four times.

**CONCLUSIONS**

The NDV isolates from pigeons appear to pose little threat to US poultry for the following reasons:

1. Chickens experimentally inoculated by natural routes did not develop clinical disease.
2. Most poultry are vaccinated and would be protected against infection with this strain of NDV.
3. The pigeon industry is aware of the problem and is starting to practice improved disease control methods including vaccination.

Precautions should be taken to insure that poultry do not become infected. To monitor the NDV situation, pigeons exhibiting clinical signs of neurotropic disease should be submitted to the laboratory for virus isolation. Infected pigeons should be kept out of contact with poultry.

**REFERENCES**

The committee met at 1:30 p.m. on October 30, 1985. A total of nineteen members and 21 guests attended.

**NEWCASTLE AND OTHER DISEASES OF IMPORTANCE**

**Newcastle Disease**

No exotic Newcastle disease was reported in the U.S. Poultry Population in FY1985. However, there were 12 isolations of velogenic viscerotropic Newcastle disease (VVND) virus and 1 isolation of velogenic Newcastle disease (VND) virus from pet birds. The VND virus was the result of a traceback to one of the VVND outbreaks. The states involved with VVND cases were:

- Arizona - 2
- California - 3
- Florida - 3
- Hawaii - 1
- Missouri - 1
- North Carolina - 1
- Puerto Rico - 1

A survey to determine whether or not VND virus is endemic in the United States was contemplated but little progress was made. Twenty-five Newcastle virus isolates from poultry from 10 states were received at the National Veterinary Services Laboratories (NVSL) for characterization. All isolates were characterized as lentogenic.

Lentogenic Newcastle disease virus has been isolated from pigeons in nine states and Canada. A more complete report of the pigeon paramyxovirus problem will be given by Dr. James E. Pearson.

**Psittacosis/Ornithosis**

In November 1984, an outbreak of ornithosis occurred in a turkey
processing plant in Virginia. There were 77 people affected in 3 processing plants. The outbreak was believed to have been caused by an infected turkey flock, but no birds were available for testing. One turkey flock with unusually high condemnations was processed at all three plants prior to the outbreak.

An initial diagnosis of ornithosis was made on a flock with turkeys in Illinois. NVSL could not confirm the diagnosis with the original material. The flock was treated with chlortetracycline for 5 days and subsequent tests were negative.

Avian Import Activities

A. Commercial Bird Program

1. There were twenty isolations of viscerotropic Newcastle disease (VVND) and velogenic Newcastle disease (VND) from infected commercial bird lots arriving from 13 countries. The countries were: Netherlands, Peru, Argentina, El Salvador, Honduras, India, Singapore, Belgium, West Germany and Guyana. These birds were refused entry into the United States. No VND or VVND was introduced into the United States from legally imported birds since 1974.

2. Approximately 575,000 commercial birds were imported through 84 USDA supervised and 3 USDA operated animal import centers. This is a decrease of almost 200,000 from FY1984. This includes 55,000 which were refused entry into the United States because of VVND virus isolations.

3. Detroit, Michigan is being closed to commercial bird imports this year. Detroit bird stations will move to Miami, Florida. Six stations from New Orleans and one from San Diego will also move to Miami, during 1986.

4. Action has been initiated to remove USDA approval to operate several stations in California, New York, and Hawaii for nonuse and failure to maintain USDA standards for approved facilities.

B. Pet Bird Program

1. Because of time and budget constraints, the U.S. Public Health Service deregulated the importation of psittacine birds in July, 1985. However, the USDA will continue to feed chlortetracycline to all psittacine birds in quarantine stations to control psittacosis.

2. There was one VVND isolation in a personal pet bird in the Miami quarantine station. Also pet bird owners are no longer limited to importing two psittacines per family per year.

C. Smuggled Bird Program

1. A new permanent smuggled bird station is now operating in Otay Mesa, California.

2. There were seven VVND isolations from seven seized lots of birds in the Otay Mesa, California, and Mission, Texas, quarantine stations.
D. Poultry and Hatching Egg Program

1. Poultry hatching egg imports for FY1985 were 4,938,978. Baby Chick Imports for FY1985 were 3,191,705.

2. In FY1986 there will be a proposal to change Title 9, Code of Federal Regulations, Part 92.11(c), Poultry Quarantine Requirements, to discontinue quarantine of poultry hatching eggs from VVND-free countries. Hatching eggs from VVND-free countries have not been quarantined since the early 1970's. Their flocks of origin are tested negative for pullorum-typhoid disease and Adenovirus 127 (egg drop syndromes). Duck and geese hatching egg flocks of origin must test negative to Derszy's disease.

Dr. I. Peterson of USDA, APHIS Veterinary Services presented a fourth draft of a "Model State Program For Poultry Disease Prevention." This program is under consideration by the National Poultry Improvement Program.

PARAMYXOVIRUS

During the last year, there have been few reports of clinical disease in poultry associated with paramyxovirus (PMV) -2 or -3 infection.

Isolations of PMV-2 were made at the National Veterinary Services Laboratories (NVSL) from a flock of 6 week old Colorado turkeys which were suffering with an avian influenza (H9N2) infection. No clinical disease was observed in turkeys inoculated with the isolate. PMV-2 isolations were also made from 6 week old California turkeys with an upper respiratory infection and from 12-week old Washington pheasants with a history of an increased mortality.

PMV-3 infections associated with a decrease in egg production were reported in a turkey flock in Virginia and there was serological evidence of infection of a Pennsylvania turkey flock prior to it starting egg production. The PMV-3 inactivated vaccine is still being used in North Carolina.

There were 13 PMD-3 isolations made at NVSL from 10 pet bird diagnostic submissions. All had a history of clinical disease. The isolations were from African frey parrots (4), parrot (1), cockatoo (3), cockatiel (3), and finches (2). There were an additional 168 isolations from pet birds that were submitted for VVNDV surveillance or trace out.

During the last year PMV-1 was identified at NVSL from pigeons on 23 premises in the following states (premises): New York (6), Maryland (5), Pennsylvania (4), Delaware (4), New Jersey (2), Virginia (1). Dr. Dennis Alexander, Weybridge England has compared the United States and British isolates and found them to be identical. Clinical disease has been reported in infected pigeons, but there has been no evidence that domestic poultry have been infected with the virus. The isolates do not produce disease in chickens inoculated by the intranasal or cloacal route. The mean death time in embryonating chicken eggs is the same as the lentogenic strains of Newcastle disease virus. However, the isolates do produce disease in pigeons inoculated by the intravenous or intramuscular route. A
significant observation has been made that the intracerebral pathogenicity index in day-old chicks is the same as for velogenic strains of Newcastle disease virus.

AVIAN INFLUENZA

Avian Influenza Eradication Program

On May 29, 1985, the U.S. Department of Agriculture officially declared lethal avian influenza (H5N2) eradicated from the U.S. The avian influenza area quarantines in Virginia and Pennsylvania were lifted September 14 and October 4, 1984, respectively. A six-month surveillance program ended in Virginia on March 15 and in Pennsylvania on April 2, 1985. During the eradication effort more than 17 million birds in 452 flocks were destroyed. The federal indemnities totaled $41.9 million with an additional $22.6 million in federal support costs, for a total cost of eradication of $64.5 million.

A report published by the National Economic Division, Economic Research Service USDA in January, 1985 reviewed the economic impact of the eradication program. In addition to the cost of $64.5 million to the U.S. government, producers suffered direct loss estimated at $55 million which were offset by the $40 million paid as indemnities. The industry had to pay additional costs due to cleanup, disinfecting, transportation, income foregone and financial hardships.

Consumers paid about $349 million more for their protein foods during November, 1983 to April 1984. Allied industries were also affected including hatcheries, feed suppliers, other supply firms, processors, distributors and credit agencies.

Without the eradication program, avian influenza outbreak would have caused much greater economic havoc. Had the outbreak spread throughout the Eastern United States, it was projected that producers would have lost an estimated $508 million and consumers would have borne an increased cost of protein foods by about $5.6 billion.

Avian Influenza in FY85

Outside the four state eradication area of Pennsylvania, Virginia, Maryland, and New Jersey, avian influenza was identified in turkey flocks in Arkansas, California, Colorado, Iowa, Michigan and Minnesota. Minnesota reported the largest number with subtypes H1N1, H2N3, H4N2, H4N6, H4N8, H6N8, H8N4. All isolations were identified by NVSL as nonpathogenic. (Table 1)

Use of Avian Influenza Vaccine

Influenza killed vaccine was used in turkeys in California (H9), Colorado (H9), Missouri (H1), Minnesota (H1, H4, H5, H6), North Carolina (H1), Ohio (H1). The vaccines were used primarily in turkey breeder flocks. No chicken flocks were reported vaccinated with AI Vaccine.
Dr. John Atwell reported that USDA, APHIS Veterinary Services is considering amending its current notice (February, 1985) regarding the use of Avian Influenza vaccines. Under the proposed amendment, conditional licensure would be granted for vaccines other than the H5 or H7 serotypes with the provision that the vaccine be used only in turkeys and only by individuals authorized by state regulatory officials. The utilization of H5 or H7 serotypes in a vaccine may be permitted subject to approval by the Deputy Administrator.

**National Veterinary Services Laboratories (NVSL)**

Dr. J. E. Pearson reported on the activities of the NVSL. From October 1, 1984 through September 30, 1985, 32,516 specimens were received for avian influenza virus isolation, 31,596 were received prior to April 1, 1985. The laboratory reported the serotyping of nonpathogenic avian influenza virus isolations from the following states:

<table>
<thead>
<tr>
<th>Month</th>
<th>State</th>
<th>Species</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>October, 1984</td>
<td>Colorado</td>
<td>Turkey</td>
<td>H9N2</td>
</tr>
<tr>
<td>November, 1984</td>
<td>Maryland</td>
<td>Guinea Fowl and Duck</td>
<td>H3N8</td>
</tr>
<tr>
<td>December, 1984</td>
<td>Arkansas</td>
<td>Turkey</td>
<td>H1N1</td>
</tr>
<tr>
<td>December, 1984</td>
<td>Virginia</td>
<td>Turkey</td>
<td>H10N8</td>
</tr>
<tr>
<td>February, 1985</td>
<td>California</td>
<td>Turkey</td>
<td>H9N2</td>
</tr>
<tr>
<td>February, 1985</td>
<td>Virginia</td>
<td>Turkey</td>
<td>N2N3</td>
</tr>
<tr>
<td>July, 1985</td>
<td>Washington</td>
<td>Pheasant</td>
<td>H9N9</td>
</tr>
<tr>
<td>September, 1985</td>
<td>Pennsylvania</td>
<td>Duck</td>
<td>H3N8</td>
</tr>
</tbody>
</table>

Minnesota turkey flocks appear to experience AI outbreaks more frequently. From October, 1984 to September, 1985, nine outbreaks involving 16 turkey flocks were identified:

<table>
<thead>
<tr>
<th>Month</th>
<th>Subtype</th>
<th>Farms</th>
<th>Flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>October, 1984</td>
<td>H8N4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>November, 1984</td>
<td>H4N6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>November, 1984</td>
<td>H1N1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>December, 1984</td>
<td>H2N3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>December, 1984</td>
<td>H1N1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>December, 1984</td>
<td>H6N8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>July, 1985</td>
<td>H6N8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>September, 1985</td>
<td>H4N2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>September, 1985</td>
<td>H4N8</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

TOTAL 9 16

In addition to the serotypes of AI viruses, identified above, serotype H7N3 was serologically detected in serums of chickens, ducks, swans and geese in Virginia in 1985.
National Veterinary Services Laboratories

Dr. J. E. Pearson submitted a report concerning Import Quarantine Station isolations (Table 2). Two AI viruses were isolated. Subserotype H9N2 was isolated from a duck in 1984 and subtype H6N2 was isolated from a spoonbill; both species were imported from France.

Poultry Disease Task Force

The Delmarva Poultry Industry developed an excellent Procedure Manual on Emergency Poultry Diseases (June, 1985). Pennsylvania has also developed an excellent Poultry Diagnostic and Monitoring Program as a cooperative program among the poultry industry, University of Pennsylvania, Pennsylvania State University, Delaware Valley College and the Pennsylvania Department of Agriculture. Minnesota has developed a cooperative control program specifically for avian influenza. These programs may serve as model programs for other states.

Dr. F. Craig emphasized in his report to the Committee on the steps they have outlined for the Delmarva area in the event of an IA outbreak. They feel that an efficient control and eradication of AI requires prompt and bold actions. These actions, however, are based on a previously proposed detailed manual of procedures which includes handling of suspect, presumptive positive and confirmed cases. Cooperation of industry groups with State and Federal authorities in the preplanning and execution phases is most important. The plans should also include sources of funding for special situations along with records of essential resources, avian species census with locations and bird movement patterns. In view of the progress made with the guidelines for controlling and eradicating AI and other infectious diseases of poultry, the subcommittee on this subject was dissolved.

RECOMMENDATION

The committee, after a brief discussion, recommended that the USDA-APHIS-USDA increase the poultry diseases budget to allow Federal Veterinary Medical Officers at the state level to be involved in field investigations of avian diseases and to allow the restaffing of regional poultry epidemiologists.

Further discussion involved the need for research with emphasis on laboratory procedures which would provide necessary information predicting the potential virulence spreading qualities, pathogenicity and economic impact on avian influenza isolates.

In view of the importance and threat of avian influenza to the United States as well as the world’s poultry industries, a second symposium on avian influenza is planned. Dr. B. Easterday, Dean of the School of Veterinary Medicine, University of Wisconsin, was named chairman with Drs. C. Beard of Athens, Georgia and D. Alexander of England as Vice Chairmen to formulate the program. The second symposium will be held on September 3, 4 and 5, 1986, in the Educational Center, University of
Georgia, Athens, Georgia. Papers and subjects to be presented are to be selected by invitation of the program committee.

MYCOPLASMOSIS

The subcommittee’s objective remains the eradication of economically significant pathogenic mycoplasmas from commercial poultry. Towards this end, six active working groups of this subcommittee have advanced the following recommendations:

1. Significant expansion of NPIP’s role beyond the breeder/hatchery level to an active role at the production levels (table egg, broiler and turkey meat flocks).
2. Further documentation of the full-range of cost/benefits associated with mycoplasma-free poultry production.
3. Strong support of research to determine the efficacy of live (F-strain and temperature-sensitive mutant) and killed MG and/or MS vaccines for (a) control of mortality and egg production losses in layers, and (b) on-farm eradication of MG and/or MS, particularly from in-line multiple-age table egg operations.
4. Development of practical diagnostic tests for the detection of M. iowae, and the standardization of enzyme-linked immunosorbent (ELISA) assays for M. gallisepticum (MG) and M. synoviae (MS), particularly those used in testing and breeding flocks for official classifications, should be addressed with urgency.

The subcommittee endorsed a resolution passed by the Northeastern Conference on Avian Diseases, calling for national standardization of ELISA tests, including mycoplasma ELISA. It further supported committee/industry collaborative development of educational materials useful to flock owners and managers interested in eradicating avian mycoplasmosis from their operation(s).

MODEL STATE PROGRAM FOR PET BIRDS

The subcommittee held two official meetings since the USAHA meeting in Fort Worth Texas, last year. The National Cage and Aviary Bird Program was amended in part to make it simpler and more flexible. During the past year, groups have evolved in several states fostering their own version of a Model State Cage and Aviary Bird Program based in part upon the basic features of the Nicabip Plan with modifications to suit their individual needs. Such plans are being examined in Maryland, Virginia, and California. Hopefully in the coming year the subcommittee may see more progress in implementing a plan.

In addition to our consideration of the Model State Program for Pet Birds, many of us were asked to consider a proposal which was made by a firm in the United States to the USDA’s Animal and Plant Health Inspection Service (APHIS) to allow pet bird parent stock originating in the United States to be relocated to a foreign-based island breeding operation and that the offspring of such stock be allowed entry into the U.S. without...
going through the customary 30 day quarantine period. The facility would not be under the direct supervision of APHIS, but only subject to one inspection every 30 days by veterinarians of the country involved.

The proposal met with considerable opposition by the U.S. poultry industries as a threat of exposure of our commercial poultry industries to exotic infectious poultry diseases. The latter was sufficient to result in a notice of withdrawal of the proposal which appeared in the August 20, 1985, Federal Register.

MISCELLANEOUS

Dr. Arthur Anderson reported that monoclonal antibodies and DNA restriction endonuclease analysis both have potential for use in differentiating strains of Chlamydia psittaci. Both techniques have shown that the turkey strains are distinct from the mammalian strains.

SANITATION

The subject of transmission of diseases of poultry through contaminated poultry hauling trucks and crates was discussed last year which resulted in appointing a subcommittee to study the various factors involved in regulations in federally inspected poultry processing plants.

After the subcommittee’s deliberation it was the opinion of the majority that no additional regulations were necessary to control transmissable disease of poultry. Each state has regulatory authority to guarantee and control movement of poultry and livestock. USDA regulatory officials are available to assist in controlling those diseases that may become a national or extraordinary emergency. Since the objectives of the subcommittee were achieved, the committee was dissolved.

The following subcommittees were formed:

**AVIAN INFLUENZA:** R. A. Bankowski, C. Beard; F. Craig; D. King; D. Halvorson; J. E. Pearson; I. Petersen; and B. S. Pomeroy, Chairperson

**MYCOPLASMOSIS:** D. Johnson; D. McMartin; E. T. Mallinson; B. S. Pomeroy; I. Peterson; W. Towers; R. Yamamoto; and H. O. Opitz, Chairperson

**MODEL STATE PROGRAM FOR PET BIRDS:** T. Angel; R. E. Baer; H. Goldstein; S. Clubb; D. J. Ligda; E. T. Mallinson; M. Myers; R. Schar; and H. Kahan, Chairperson

**PARAMYXOVIRUS EVALUATION:** C. Beard; I. H. Kahan; D. King; C. Weston, R. A. Bankowski; and J. E. Pearson, Chairperson
TABLE 1
AVIAN INFLUENZA SEROTYPES ISOLATED FROM TURKEYS, CHICKENS AND OTHER DOMESTIC FOWL IN THE U.S. (1964–1985) OR BASED ON SEROLOGY

<table>
<thead>
<tr>
<th>STATE</th>
<th>Turkeys</th>
<th>Year First Identified</th>
<th>Hemagglutinin Antigens Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>1964</td>
<td>H5, H6, H9</td>
<td></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>1965</td>
<td>H6</td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1965</td>
<td>H5, H6, H9</td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>1966</td>
<td>H1, H2, H3, H4, H5</td>
<td>H6, H7, H8, H9, H10</td>
</tr>
<tr>
<td>Washington</td>
<td>1967</td>
<td>H6</td>
<td></td>
</tr>
<tr>
<td>Oregon</td>
<td>1970</td>
<td>H6, H7</td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>1971</td>
<td>H1, H4, H5, H6</td>
<td></td>
</tr>
<tr>
<td>Colorado</td>
<td>1972</td>
<td>H1, H5, H9</td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>1975</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>South Dakota</td>
<td>1978</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>1979</td>
<td>H5, H7, H9</td>
<td></td>
</tr>
<tr>
<td>Missouri</td>
<td>1980</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>Kansas</td>
<td>1980</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>North Dakota</td>
<td>1981</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>Arkansas</td>
<td>1981</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>1982</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H5, H10, H2</td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>H5, H1</td>
<td></td>
</tr>
<tr>
<td>Michigan</td>
<td>1985</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>Chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alabama</td>
<td>1975</td>
<td>H4</td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>1978</td>
<td>H6</td>
<td></td>
</tr>
<tr>
<td>District of Columbia</td>
<td>1980</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>Maryland</td>
<td>1983</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>New Jersey</td>
<td>1983</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>1983</td>
<td>H5, H7</td>
<td></td>
</tr>
<tr>
<td>Other Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1969</td>
<td>Ducks NA, H3</td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>1974</td>
<td>Geese NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>Guinea Fowl NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>Pheasants, H3, H7</td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>1978</td>
<td>Ducks H3, H4, H5, H6 H11</td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1983</td>
<td>Guinea Fowl, Quail H5</td>
<td></td>
</tr>
<tr>
<td>Maryland</td>
<td>1984</td>
<td>Ducks, Guinea Fowl H5</td>
<td></td>
</tr>
<tr>
<td>Washington</td>
<td>1985</td>
<td>Pheasant H9</td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>1985</td>
<td>Ducks, Swans, Geese H7</td>
<td></td>
</tr>
<tr>
<td>Maryland</td>
<td>1984</td>
<td>Chukar, H5</td>
<td></td>
</tr>
</tbody>
</table>

NA — NOT AVAILABLE
<table>
<thead>
<tr>
<th>Specimens:</th>
<th>1984</th>
<th>1985 thru Sept 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lots — Private Facilities</td>
<td>257</td>
<td>220</td>
</tr>
<tr>
<td>Private Facilities</td>
<td>32,306</td>
<td>28,081</td>
</tr>
<tr>
<td>USDA Facilities</td>
<td>3,931</td>
<td>1,454</td>
</tr>
<tr>
<td>Lots of HAV</td>
<td>69</td>
<td>**</td>
</tr>
<tr>
<td>HAV isolates</td>
<td>935</td>
<td>1,106 *</td>
</tr>
<tr>
<td>Influenza positive lots</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>PMV-2 percent of HAV isolates</td>
<td>32.8</td>
<td>59.9</td>
</tr>
<tr>
<td>PMV-3 percent of HAV isolates</td>
<td>38.5</td>
<td>18.8</td>
</tr>
<tr>
<td>VVNDV positive lots — Private Facilities</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>VNDV positive lots — Private Facilities</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>VVNDV positive lots — USDA Facilities</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>VVNDV positive lots — confiscated birds</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Lentogenic NDV positive lots — USDA Facilities</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lentogenic NDV positive lots — Private Facilities</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

HAV — Hemagglutinating viruses other than Newcastle disease virus  
VVNDV — Velogenic viscerotropic Newcastle disease virus  
VNDV — Velogenic Newcastle disease virus  

* — Typing of the HAV isolate has not been completed.  
** — Not available.  
*** — H9N2 was isolated from a duck in 1984. In 1985, H6N2 was isolated from a spoonbill; both were imported from France.
REPORT OF THE COMMITTEE ON PSEUDORABIES

Chairman: Dr. M. H. Lang, Des Moines, IA
Vice Chairman: Mr. P. E. Bradshaw, Griggsville, IL

Fred Aldernik, MD; George Edwards, NC; D. D. Gingerich, IA; Howard T. Hill, IA; L. W. Hinchman, IN; Donald Hoogestraat, SD; C. L. Kanitz, IN; John P. Kluge, IA; James P. Quigley, Jr., GA; Carson Rogers, NE; L. W. Schnurrenberger, MD; Ed Slauter, MO; Paul L. Spencer, IL; Thomas Thurber, NE; Williard Waldo, NE.

The meeting was called to order at 1:30 p.m., October 30, 1985 by the chairman, Dr. M. H. Lang, with 18 members and about 75 guests present.

Dr. George Beran of Iowa State University presented a review of the pilot projects, concluding with the comment: "We have learned a great deal; the day is past when we say 'if' we can control and eliminate pseudorabies; the most important question remaining is when."

Preliminary economic analyses of the pilot projects were presented by John Ambrosius, University of Wisconsin, and Arne Hallem, Iowa State University. Hallem indicated that the data available on Iowa herds indicates that one to 1.5% of herds in Iowa would experience a clinical outbreak each year at an estimated loss of six to eight million dollars.

Richard L. Sharpee of Norden Laboratories reported that the sub-unit vaccine, not yet on the market, is equivalent in efficacy to the Norden modified live product and it has been demonstrated that it can differentiate between vaccinated pigs and those exposed to the field viruses. He said the company hopes to have the vaccine on the market next summer.

Alec Hogg of Nebraska, reporting for Therese Brown, indicated research on runoff water from lots containing clinically ill hogs is probably not a source of virus to other farms.

Beran reported for Howard Hill of Iowa State University on research on the latex agglutination test. He said the LA test appears to be more sensitive than the SN test, detecting antibodies sooner than the SN test, and that results of the LA test and the ELISA test are closely correlated. Hill found hogs positive to the LA test that were SN negative in herds with SN positive hogs. There were no false negatives, he reported.

Preston Dorsett of Viral Antigens, developer of the LA test, said it is being studied in Nebraska, Ohio, Indiana and Arkansas in addition to Iowa. Initial results on use by field veterinarians have been good. The company is in the process of completing an application for licensure of the test and will ask for a license for use by approved labs. Asked about the availability of the license for use in herds by practicing veterinarians, Dorsett said the application will not preclude that use.

Mike Snyder of Agri-Tech Systems reported on research on differentiation between IgM and IgG antibodies in hogs challenged with PRV,
indicating the technique may provide a method for determining the date of infection more accurately.

Neal Black of LCI reported on development of criteria for designating low-prevalence areas, as drafted by a sub-committee of the pseudorabies committee of Livestock Conservation Institute. He said the sub-committee recommended that the criteria be a voluntary industry program sanctioned by the states, with state-area status determined by a joint committee of USAHA, LCI and the National Pork Producers Council. One sentence of the report referring to authorities for states or areas entering the preliminary phase of the program (page 1, line 23), was amended to read:

"2. Authority to quarantine feeder pigs of unknown status."

On motion, seconded and carried by the committee members present, the criteria were accepted as amended and referred to the industry group as defined in the report.

Speaking on "Where Do We Go From Here?" were David Meisinger of the National Pork Producers Council, Dr. Ralph Vinson of Illinois, Dr. L. W. Schnurrenberger of APHIS, and Dr. David Thawley of the University of Missouri.

Meisinger called attention to an industry symposium being planned for January 20–21 in Peoria, IL as a followup to the pilot projects which will attempt to chart a future course with regard to the disease. Vinson supported an eradication program as the only practical alternative for the future. Schnurrenberger said he expects final publication of revised PRV regulations soon. Thawley listed research needs in the following areas; feral swine, latency, rapid field tests, sub-unit vaccines and identification. He declared that it is not up to the scientists to determine future actions with respect to the disease; "it's in the hands of the industry."
PRV-FREE STATUS CRITERIA
Neal Black, St. Paul, MN
Philip Bradshaw, Griggsville, IL

Summary

The purpose of these criteria is to establish a method by which a state or area can be demonstrated to have a very low prevalence or no pseudorabies (PRV). It is not an eradication program. It is intended for use in states or areas which have either eradicated PRV or have demonstrated a very low or no prevalence of the disease. The term free is not used in the criteria by design, since the surveillance procedures are not designed to establish absolute freedom of the area with regard to PRV. Rather, they are designed as a means of demonstrating that animals from such an area are an extremely low risk with regard to transmission of PRV. It is expected that swine, either breeding stock or feeder pigs, could move within and out of such an area without restriction or testing requirements as far as PRV is concerned.

This is intended as a voluntary industry program sanctioned by the states, with state/area status determined by a joint committee of LCI, NPPC and USAHA.

The criteria include two classes: Class B for states or areas attempting to demonstrate that PRV probably does not exist through one of the two optional surveillance procedures outlined, and Class A for states which have carried out the procedures in Class B and are continuing to monitor to determine if reinfection occurs through procedures outlined here. Before entering Class B a state or area must demonstrate authorities and abilities as follows:

1. Pseudorabies is a reportable disease, authority for quarantine of confirmed cases and a procedure for release of quarantine.
2. Authority to quarantine feeder pigs of unknown status.
3. Surveillance at slaughter or first point of sale, including tracing positives to farm of origin and a plan for dealing with positive herds.
4. An effective identification system for cull sows and boars.

Class B

A. Surveillance options—perform one of the following:

1. Monitoring of cull breeding stock
   (a) Test one-third of cull breeding stock sent to slaughter, either at slaughter or first point of sale, for two years, with traceback of 80% of positives to farm of origin over the two-year period, or test sufficient cull breeding stock either at slaughter or first point of sale, for two years, at rates of testing and traceback sufficient to provide a “traceback capability” of 25%. “Traceback capability” is determined by multiplying the percentage of the population sampled by the percentage of reactors traced. For
purposes of this provision, the percentage of cull breeding stock tested will be determined by dividing the number tested by an estimate of the total breeding stock available for testing based on multiplying the USDA Crop Reporting Board estimate of sows farrowing in the state or area by a 30% annual culling rate.

(b) The state shall require identification of sows and boars to farm of origin.

(c) No new confirmed cases detected during the second year of the testing period except those which it can be demonstrated resulted from importation from outside the area and from which there was no spread to other herds. A confirmed case is defined as a herd in which epidemiological evidence, including testing of animals remaining in the herd, has confirmed the presence of positive PRV titers in the herd. Until such confirmation is obtained, a herd for which a positive test result has been obtained in the slaughter survey will be considered a suspect herd.

(d) No infected/quarantined herds remaining in the area at the end of the period. Any infected/quarantined herds which may have existed in the area at the beginning of the period have been cleaned up, along with any infected herds disclosed in (c).

2. Monitoring of herds

(a) Test every herd in the state/area during no more than one year, the sampling procedure to be that being used in the pilot projects as follows:

(1) Herd of less than 100 adult breeding animals, test all breeding animals up to 25 head.
(2) Herd of 100 to 200 adult breeding animals, test 27 breeders.
(3) Herd of more than 200 adult breeding animals, test 29.
(4) Plus all additions to the herd within the previous 6 months.
(5) Tested swine to be selected at random and include all herd boars, all groups to be proportionately represented.

(b) No new confirmed cases unless it can be demonstrated that there has been no spread to other herds.

(c) No infected/quarantined herds remaining in the area at the end of the period. Any infected/quarantined herds which may have existed in the area at the beginning of the period have been cleaned up, along with any infected herds disclosed in (b).

B. Feeder Pigs

A program is conducted to monitor all feeder pig finishing herds in the area and establish negative status. Such a program could involve testing a sample of pigs in each feeding floor, the sample size to be determined on the same basis as in 2 (a) (1), (2) and (3) above, or a slaughter hog surveillance program which includes feeder pig finishing herds.
C. Vaccination

Vaccination prohibited except in herd clean-up efforts with permission of the state veterinarian or use of sub-unit vaccines on approval of the state veterinarian.

D. Quarantines

Quarantines will be imposed and lifted per federal regulations as outlined in Part 85.1 (1), for which a proposed amendment was published April 16, 1985.

E. Controls on importations into Class B states/areas

1. Breeding stock imported into the area must be from either
   (a) A Class A area
   (b) A qualified free herd
   (c) On the basis of an individual animal negative test, a mandatory isolation and negative retest.

2. Feeder pigs imported into the area must be from either
   (a) A Class A area
   (b) A qualified Feeder Pig Herd, which is a feeder pig production herd sampled, tested and determined not to be infected annually, the sampling level to be as follows:
      (1) Herd of less than 100 adult breeding animals, test all animals up to 25 head.
      (2) Herd of 100 to 200 adult breeding animals, test 27.
      (3) Herd of more than 200 adult breeding animals, test 29.
      (4) Plus all additions to the herd within the previous 6 months.
      (5) Tested swine to be selected at random and include all herd boars, all groups to be proportionately represented.
   (c) From another Class B state/area.
   (d) From any source if the feeder pigs test negative.
   (e) If feeder pigs of unknown status are imported into a Class B state under quarantine to slaughter the herd of destination shall be treated as any other quarantined herd and it must be demonstrated that the herd is negative or has been sold out at the end of the qualification surveillance period.

Class A Status

1. Successful completion of one of the surveillance options outlined above for Class B status.

2. Surveillance of cull breeding stock for one year either at slaughter or first point of sale as provided in Class B, surveillance option A, section 1, monitoring of cull breeding stock: test one-third of cull breeding stock with traceback of 80% of positives, or a “traceback capability” of 25%; continued surveillance thereafter of cull breeding stock either at slaughter or first
point of sale at a sufficient rate to provide a traceback capability, as defined in Class B, Option A, Section 1, of 5%.

3. No confirmed cases.

In the event of a confirmed case in a Class A state, status will be suspended until 60 days after the last confirmed case has been cleaned up.

4. Vaccination restrictions and quarantines as outlined for Class B.

5. Importations of breeding stock from:
   (a) Another Class A area
   (b) A qualified free herd
   (c) Upon test, mandatory isolation and negative retest.

6. Importations of feeder pigs from:
   (a) Another Class A area
   (b) A Qualified Feeder Pig Herd as outlined above.
   (c) All pigs test negative.
THE FIVE PSEUDORABIES PILOT PROJECTS
George W. Beran
College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011

Four times in the past decade, USDA/APHIS coordinated national serological surveys of slaughter swine to assess the prevalence of pseudorabies (PR) state by state. The findings for the total United States showed a steady increase from 0.56% in 1974 to 8.78% in 1984. For the five project states, Illinois and Iowa are high prevalence states and North Carolina, Pennsylvania, and Wisconsin are relatively low prevalence states. See Figure 1.

In early 1983, the National Pork Producer’s Council (NPPC) proposed to APHIS that pilot projects be conducted in several states with joint financial sponsorship of NPPC and APHIS. The objectives as set forth by the NPPC are shown in Figure 2.

Five pilot projects were proposed by Illinois in Pike and Macoupin Counties, by Iowa in Marshall County, and as part of state eradication programs in North Carolina, Pennsylvania and Wisconsin. The areas included are shown in Figure 3.

A Technical Advisory Committee was appointed by NPPC and APHIS to oversee the technical functioning of the pilot projects. Each state set its own objectives with discussion and approval of the Technical Advisory Committee. The objectives as approved for implementation gave a diversity of approaches with a common goal of pseudorabies elimination on area bases as shown in Figures 4 through 8.

Project plans were drawn up by each pilot state and approved by the Technical Advisory Committee. All based herd clean ups on the Livestock Conservation Institute (LCI) Guidelines.

Illinois has used farm to farm sampling in a voluntary program with quarantine of all infected herds, vaccination by permit only and most herd clean ups by depopulation as shown in Figure 9.

Iowa has also used farm to farm sampling for case finding with essentially all swine farmers in the county voluntarily participating. Vaccination has been encouraged in female breeding stock in infected and high risk herds. Most herd clean ups have been by offspring segregation. See Figure 10.

North Carolina, in a statewide project has used slaughter sampling with traceback and quarantine of all infected farms. Vaccination has been by permit and most clean ups have been by test and removal. The project began in February 1984 and is still in the early states of area clean up. See Figure 11.

The Pennsylvania project is statewide but has largely been centered in the Lancaster County focus of pseudorabies. Case finding has been by
slaughter sampling with trace back and quarantine of infected farms with shipment from infected herds to slaughter only under permit. Herd clean ups have been principally by depopulation and must be completed within 8 months following quarantine. See Figure 12.

The Wisconsin project has been incorporated into the state eradication program initiated in 1976. Case finding has been by testing of hogs at all markets with trace back and quarantine of all infected herds, a two year time limit for herd clean up, principally by depopulation. See Figure 13.

The status of pseudorabies in the pilot areas at the start of the projects is shown in Figure 14. The herds which have been identified since initiation of the projects are shown in Figure 15. Finally, the progress toward area elimination of pseudorabies in each pilot area as of September 30, 1985 is shown in Figure 16.

All pilot project states have made progress in meeting the objectives of their projects. Illinois and Iowa are projecting forward to expand their programs into statewide elimination efforts and North Carolina, Pennsylvania and Wisconsin are projecting achievement and maintenance of pseudorabies free status. The pilot projects have already shown that area clean up of pseudorabies is possible and that several methods can be effectively applied in achieving effective area control. What the pilot projects cannot tell us is when we are going to move toward effective national control. Only we can decide this. See Figure 17.

FIGURE 1
PSEUDORABIES SEROLOGICAL SURVEYS
ON SLAUGHTER SWINE
Percent Positive by Serum Neutralization Tests

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total U.S.:</td>
<td>.56%</td>
<td>3.73%</td>
<td>8.39%</td>
<td>18.80%</td>
</tr>
<tr>
<td>Pilot Project States:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois:</td>
<td>1.14</td>
<td>3.29%</td>
<td>6.40%</td>
<td>17.05%</td>
</tr>
<tr>
<td>Iowa:</td>
<td>.55</td>
<td>5.82%</td>
<td>13.04%</td>
<td>34.29%</td>
</tr>
<tr>
<td>North Carolina:</td>
<td>0</td>
<td>3.44%</td>
<td>6.45%</td>
<td>0</td>
</tr>
<tr>
<td>Pennsylvania:</td>
<td>2.20</td>
<td>1.59%</td>
<td>10.53%</td>
<td>6.25%</td>
</tr>
<tr>
<td>Wisconsin:</td>
<td>.52</td>
<td>1.41%</td>
<td>2.96%</td>
<td>4.76%</td>
</tr>
</tbody>
</table>

Figure 2.
PURPOSES OF PSEUDORABIES PILOT PROJECT
National Pork Producers Council

A. Pilot projects should be designed to determine the practicality of area eradication of pseudorabies.
B. Pilot projects should be designed to provide definite answers as to whether pseudorabies eradication is achievable.
C. Government and producers must accept the results of the projects and modify approaches as necessary.
D. A technical advisory committee should decide technical aspects of pilot project designs.
Figure 3.
STATE PSEUDORABIES PILOT PROJECTS
Figure 4.

GOAL OF THE IOWA PSEUDORABIES PILOT PROJECT

To test the feasibility of controlling and/or eliminating pseudorabies in herds in an endemic area. It may also demonstrate a method for preparing an epidemic area to initially control pseudorabies leading to its elimination. The methods used are based on voluntary participation of swine producers in the project area.

Figure 5.

THE ILLINOIS PSEUDORABIES PILOT PROJECT

I. Objective:
   To eradicate pseudorabies from pilot project areas of Pike and Macoupin Counties.

II. Subobjectives:
   A. To determine the means of PR spread to herds within the area and what additional measures, if any are necessary to control PR spread.
   B. To determine if the project can control PR if participation by herd owners is voluntary.
   C. To further determine the effectiveness of the three herd cleanup strategies.
   D. To evaluate the skin test.

Figure 6.

THE NORTH CAROLINA PSEUDORABIES PILOT PROJECT

I. Objective:
   To control/eradicate pseudorabies in North Carolina.

II. Subobjectives:
   A. To determine if testing of sow and boar blood samples collected at abattoirs in North Carolina is an effective surveillance method.
   B. To determine if state authorities are adequate to control/eradicate PR or what additional authority is required. Particular emphasis will be given to:
      1. Swine identification.
      2. Herd cleanup.
      3. Determination of the technical feasibility of PR control/eradication.

Figure 7.

THE PENNSYLVANIA PSEUDORABIES PILOT PROJECT

I. Objective:
   To control/eradicate pseudorabies in Pennsylvania.

Figure 8.

THE WISCONSIN PSEUDORABIES PILOT PROJECT

I. Objective:
   To eradicate pseudorabies from Wisconsin.

II. Subobjectives:
   A. To evaluate different PR surveillance techniques.
   B. To determine infection rates of Wisconsin farms and pigs on infected Wisconsin farms.
   C. To determine the means of PR spread to herds within the state and additional measures, if any, necessary to control spread.
   D. To determine the effectiveness of different clean-up strategies.
Figure 9.

PROJECT PLANS IN THE PILOT STATES — ILLINOIS

Initiation: April 1983.
Area: 5 townships in Macoupin County, 3 townships in Pike County.
Enrollment: Voluntary.
Case Finding: Herd sampling farm to farm.
Serosampling: Regulatory and practicing veterinarians.
Quarantine: All infected herds.
Herd Plans: Based on LCI Guide. Most cleanup by depopulation.
Vaccination: By permit in infected herds only.
Skin Test: Incorporated in case finding with limited success.

Figure 10.

PROJECT PLANS IN THE PILOT STATES — IOWA

Initiation: July 1983.
Area: Marshall County.
Enrollment: Voluntary — 99% participating.
Case Finding: Statistical herd sampling farm to farm.
Serosampling: Practicing veterinarians.
Quarantine: All infected herds.
Herd Plans: Based on LCI Guide. Most by offspring segregation.
Vaccination: Encouraged in sows in infected and high-risk herds.
Subunit Vaccine: Field tests incorporated into project.
Latex Agglutination: Comparison of this serological test on field sera.

Figure 11.

PROJECT PLANS IN THE PILOT STATES — NORTH CAROLINA

Area: Statewide eradication program.
Enrollment: All infected herds quarantined until cleaned up.
Case Finding: Slaughter samples with traceback.
Serosampling: Statistical sampling of traceback and related herds by regulatory personnel or practicing veterinarians.
Quarantine: All infected farms. Slaughter only at approved plants.
Herd Plans: Based on LCI Guide. Most by test and removal.
Vaccination: Discouraged except with permit.

Figure 12.

PROJECT PLANS IN THE PILOT STATES — PENNSYLVANIA

Initiation: October 1983.
Area: Incorporated in state eradication plan initiated in 1980.
Enrollment: Mandatory cleanup of all infected herds.
Case Finding: Slaughter testing of all sows and boars with traceback.
Serosampling: All traceback and related herds by regulatory veterinarians.
Quarantine: All infected herds. Ship direct to slaughter by permit only.
Herd Plans: Based on LCI Guide. Must be completed within 8 months.
Vaccination: Prohibited except by permit.
DNA Fingerprinting: Being studied for epidemiological tracing.
THE FIVE PSEUDORABIES PILOT PROJECTS

Figure 13.

PROJECT PLANS IN THE PILOT STATES — WISCONSIN

Area: Incorporated in state eradication program initiated in 1976.
Enrollment: Mandatory cleanup of all infected herds.
Case Finding: Swine identification and testing at all markets.
Serosampling: All traceback and related herds by regulatory veterinarians.
Quarantine: All infected herds. Indemnity is paid on herd plan.
Herd Plans: Based on LCI Guide. 2-year time limit for cleanup.
Vaccination: Prohibited except by permit.

Figure 14.

AREAS AND INITIAL STATUS OF PSEUDORABIES IN PROJECT STATES

<table>
<thead>
<tr>
<th>State</th>
<th>Area Include in Projects:</th>
<th>Farms Infected at Project Start:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>171 herds, Macoupin and Pike Cos.</td>
<td>33 identified in initial survey</td>
</tr>
<tr>
<td>Iowa</td>
<td>220 herds, Marshall County</td>
<td>31 identified in initial survey</td>
</tr>
<tr>
<td>N. Carolina</td>
<td>Statewide, most herds in east</td>
<td>86 infected herds at start</td>
</tr>
<tr>
<td>Penn.</td>
<td>Statewide, most herds in southeast</td>
<td>11 quarantined herds at start</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>Statewide, most herds in southeast</td>
<td>13 quarantined herds at start</td>
</tr>
</tbody>
</table>

Figure 15.

TESTING AND IDENTIFICATION OF INFECTED HERDS DURING PROJECTS

<table>
<thead>
<tr>
<th>State:</th>
<th>Herds Tested After Project Initiated</th>
<th>Breeding Stock Slaughter Sampled</th>
<th>Herds Identified At Infected During Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>233</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Iowa</td>
<td>737</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>North Carolina</td>
<td>479</td>
<td>6,838</td>
<td>92</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>83</td>
<td>22,613</td>
<td>76</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>62</td>
<td>21,049</td>
<td>18</td>
</tr>
</tbody>
</table>

Figure 16.

PROGRESS IN PSEUDORABIES ELIMINATION IN PROJECT STATES

<table>
<thead>
<tr>
<th>State:</th>
<th>Infected Premises</th>
<th>Cleanup Completed</th>
<th>Method of Cleanup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% Depopulation</td>
<td>Test &amp; Removal</td>
</tr>
<tr>
<td>Illinois</td>
<td>36</td>
<td>22</td>
<td>61</td>
</tr>
<tr>
<td>Iowa</td>
<td>40</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>N. Carolina</td>
<td>111</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Penn.</td>
<td>87</td>
<td>74</td>
<td>68</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>31</td>
<td>13</td>
<td>42</td>
</tr>
</tbody>
</table>

Figure 17.

DEVELOPMENTS SINCE INITIATION OF PILOT PROJECTS

Illinois:
- Feeder pig certification initiated February 1985.
- Movement of feeder pigs from quarantined herds to end July 1986.
- Illinois Pseudorabies Control Act is being developed. To be implemented 1987.
- Marked sow and boar testing planned.

Iowa:
- Regulation of all swine movement planned.
- Plan farm to farm case finding with mandatory cleanup.

North Carolina, Pennsylvania, Wisconsin:
- Completion of cleanup of infected herds.
- Achievement and maintenance of free status.
COST/BENEFITS OF PSEUDORABIES ERADICATION IN WISCONSIN

by
Dr. John Ambrosius
Professor of Farm Management
University of Wisconsin-Platteville
Platteville, Wisconsin

In February, 1984, Wisconsin passed legislation to eradicate pseudorabies. The law was passed by a large majority in both the house and senate. This was achieved due to the efforts of Wisconsin Pork Producers and other livestock commodity groups.

A limited number of infected swine herds exist in Wisconsin. On October 1, 1985 there were 15 infected herds. Since December 1976, there has been 60 total cases.

The purpose of this project was to analyze the cost and benefits in Wisconsin of eradicating pseudorabies.

Specific objectives were:

1. to obtain the private and public costs associated with a pseudorabies eradication program in Wisconsin.

2. To assess the public and private benefits of a pseudorabies eradication program in Wisconsin. These benefits would be the savings in costs to:

   a. swine producers by avoiding pseudorabies infection to their swine herds
   b. swine producers by needing less preventive measures to keep pseudorabies out of their herds
   c. other livestock producers by preventing transmission of pseudorabies to their livestock
   d. the public sector.

A questionnaire was developed and pretested. It was used to collect the necessary data with a personal interview. The questionnaire included general swine herd information and eradication cost sections. The general swine herd information section determined the type of swine production system and size of the swine herd. Eradication costs include labor and materials to clean up, costs to isolate clean stock, loss in value of breeding stock or feeder pigs, veterinarian costs, opportunity cost of down time and start up costs with clean foundation stock.

Public cost data were obtained through personal visits with the personnel of Animal Health Division in Wisconsin Department of Agriculture, Trade and Consumer Protection and with personnel of the Wisconsin Area Office of Veterinary Service, APHIS.

The second objective was to assess the benefits of pseudorabies eradi-
COST/BENEFITS OF PSEUDORABIES ERADICATION

This information was calculated using costs and other losses were experienced when pseudorabies infection existed. These costs and losses will serve as a basis to derive benefits of pseudorabies eradication.

Part 2a were collected in connection with the farm questionnaire in objective one. Cost data collected included such items as death losses, reduced gains, poor feed conversion, veterinary costs, losses in selling feeder pigs and breeding stock plus additional marketing costs experienced by producers with infected herds.

Data for objective 2b and 2c were obtained by selecting a random sample of swine and other livestock producers in the immediate area of the infected herds. A pretested questionnaire was used with a personal visit to collect the data. The data gathered included costs to those producers in attempting to prevent pseudorabies infection or reinfection. These costs would include such items as pseudorabies testing, extra labor time from cleaning, restriction of persons, trucks, machinery, plus movement of grain and animal wastes.

Objective 2d was obtained from personnel in both the Animal Health Division of Wisconsin Department of Agriculture, Trade and Consumer Protection and the Wisconsin Area Office of Veterinary Services, APHIS, to determine any public costs associated with eradicating pseudorabies from livestock.

A total of 27 usable farm questionnaires were obtained. Four swine producers refused to cooperate in the survey.

Twenty-four of the operations were farrow to finish. Some of these operators had been involved in other types of operation prior to contracting pseudorabies infections. To minimize losses they converted to farrow to finish operations. There were one seedstock producer, feeder pig producer and feeder pig finisher (see Table I).

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedstock Producer</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>Farrow to finish</td>
<td>24</td>
<td>88.9</td>
</tr>
<tr>
<td>Feeder pig producer</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>Feeder pig finisher</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>27</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table II shows the types of facilities used by the 27 cooperating producers. Most producers used a combination of facility types. Eight producers used only one type of facility.
Table II
Type of Facilities

<table>
<thead>
<tr>
<th>Type(^a)</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>AB</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>ABC</td>
<td>3</td>
<td>11.1</td>
</tr>
<tr>
<td>ABCD</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>AC</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>BC</td>
<td>5</td>
<td>18.6</td>
</tr>
<tr>
<td>BCD</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>BD</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>CD</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>CD</td>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\(^a\) A = Total confinement  
B = Partial confinement  
C = Open front building  
D = Pasture  
E = Other

Table III presents the swine inventory of producers as of the date of the first pseudorabies test. The herds averaged nearly 77 sows.

Table III
Swine Inventory on Date of First Pseudorabies Test

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sows and gilts</td>
<td>76.89</td>
</tr>
<tr>
<td>Boars</td>
<td>3.55</td>
</tr>
<tr>
<td>Growing/finishing pigs</td>
<td>481.92</td>
</tr>
<tr>
<td>Pigs birth to weaning</td>
<td>184.61</td>
</tr>
<tr>
<td>Litter size at birth</td>
<td>10.13</td>
</tr>
<tr>
<td>Pigs weaned per sow</td>
<td>8.45</td>
</tr>
<tr>
<td>Hogs sold per litter</td>
<td>7.65</td>
</tr>
<tr>
<td>Number pigs tested</td>
<td>23.72</td>
</tr>
<tr>
<td>Number pigs positive</td>
<td>11.17</td>
</tr>
</tbody>
</table>

Table IV
Labor Used and Cost on Date of First Pseudorabies Test

<table>
<thead>
<tr>
<th>Item</th>
<th>Hours</th>
<th>Value/hour</th>
<th>Total Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operator</td>
<td>2.77</td>
<td>$7.93</td>
<td>$21.97</td>
</tr>
<tr>
<td>Veterinary</td>
<td>—</td>
<td>—</td>
<td>14.81</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>$36.78</td>
</tr>
</tbody>
</table>
Labor cost on the date of the first pseudorabies test was $36.78. Farm operators used 2.77 hours of time with an estimated labor value of $7.93 per hour for a total of $21.97. Veterinary costs were $14.81 (Table IV).

**Table V**

Costs of Remaining Pseudorabies Free

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing</td>
<td>$21.51</td>
</tr>
<tr>
<td>Labor to guarantee</td>
<td>48.65</td>
</tr>
<tr>
<td>Facility maintenance</td>
<td>133.33</td>
</tr>
<tr>
<td>Vaccine</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$203.49</strong></td>
</tr>
</tbody>
</table>

*Period of three months.*

Swine producers free of pseudorabies infection estimated costs of $203.49 to avoid reinfection. Maintenance of facilities was the major cost of $133.33 per producer. Labor costs was the next highest cost of $48.65 (Table V).

Clinical outbreaks in pseudorabies infected swine herds were very minimal. Table VI shows the losses due to clinical outbreaks from pseudorabies infection. The average for each producer was 2.26 sows aborting and 50 stillborn pigs. Nearly 60 nursing pigs per producer were lost due to pseudorabies infection. Most producers had no clinical outbreaks. All of the losses occurred on only a few farms.

**Table VI**

Losses From Clinical Outbreaks Due to Pseudorabies Infection

<table>
<thead>
<tr>
<th>Item</th>
<th>Pseudorabies Net Percent Present</th>
<th>Absence Net Percent Present</th>
<th>Pseudorabies Net Percent Present due to Pseudorabies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sows aborting</td>
<td>2.26</td>
<td>4.74</td>
<td>4.74</td>
</tr>
<tr>
<td>Stillborn pigs</td>
<td>50.23</td>
<td>9.24</td>
<td>8.09</td>
</tr>
<tr>
<td>Nursing pigs died</td>
<td>59.59</td>
<td>21.74</td>
<td>12.22</td>
</tr>
<tr>
<td>Growing pigs died</td>
<td>1.93</td>
<td>.81</td>
<td>0.52</td>
</tr>
<tr>
<td>Backward pigs</td>
<td>6.15</td>
<td>3.33</td>
<td>2.33</td>
</tr>
<tr>
<td>Sows open</td>
<td>3.20</td>
<td>---</td>
<td>4.17</td>
</tr>
</tbody>
</table>

Direct swine producer costs from pseudorabies infection over 25.37 months was $5081.24. Major losses were in reduced seedstock sales—$1792.59 and feeder pig sales—$1422.22. Death losses and stillborn pigs accounted for over $1080 of losses. Aborting sows and open sows contributed to $363 of losses. Some losses due to deaths of other livestock on the farm. The average loss was $365. Some producers initially attributed death losses to pseudorabies infection when the real cause of death loss were other factors.
Swine producers incurred $13,168 in costs to eradicate pseudorabies (Table VIII). The major cost was losses from down time. This accounted for $5785. Marked losses due to depopulation of the herd—$2350 and re-population of the herd—$2683 were the next highest costs. Labor costs of segregating the herd and cost of maintaining segregated facilities were $526 and $522 respectively.

Most producers preferred to live with pseudorabies infection rather than eradicate pseudorabies infection. They preferred to vaccinate to avoid clinical outbreaks and to live with the infection.

Table VII
Direct Costs of Pseudorabies Infection

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sows aborting</td>
<td>$165.04</td>
</tr>
<tr>
<td>Stillborn pigs</td>
<td>251.15</td>
</tr>
<tr>
<td>Nursing pigs died</td>
<td>834.26</td>
</tr>
<tr>
<td>Growing pigs died</td>
<td>84.92</td>
</tr>
<tr>
<td>Backward pigs</td>
<td>43.96</td>
</tr>
<tr>
<td>Sows open</td>
<td>198.29</td>
</tr>
<tr>
<td>Seedstock sales</td>
<td>1792.59</td>
</tr>
<tr>
<td>Feeder pig sales</td>
<td>1422.22</td>
</tr>
<tr>
<td>Other livestock</td>
<td>364.81</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$5081.24</strong></td>
</tr>
</tbody>
</table>

\( ^a \) Costs over 25.37 months

Table VIII
Producer Costs of Eradicating Pseudorabies

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinating</td>
<td>$0</td>
</tr>
<tr>
<td>Market Loss Positive Swine Sold</td>
<td>275.26</td>
</tr>
<tr>
<td>Labor Costs for Blood Tests</td>
<td>63.12</td>
</tr>
<tr>
<td>Labor Costs of Segregating Herd</td>
<td>526.24</td>
</tr>
<tr>
<td>Cost of Maintaining Segregating Facilities</td>
<td>522.22</td>
</tr>
<tr>
<td>Market Loss to Depopulate Herd</td>
<td>2349.67</td>
</tr>
<tr>
<td>Repopulate Herd</td>
<td>2688.15</td>
</tr>
<tr>
<td>Cleaning Supplies</td>
<td>355.19</td>
</tr>
<tr>
<td>Labor Cost of Cleaning</td>
<td>136.24</td>
</tr>
<tr>
<td>Labor Cost Testing Herd Additions</td>
<td>21.81</td>
</tr>
<tr>
<td>Cost of Isolation Herd Additions</td>
<td>455.02</td>
</tr>
<tr>
<td>Losses from Down Time</td>
<td>5785.23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$13,168.15</strong></td>
</tr>
</tbody>
</table>
A survey of Lafayette county pork producers was conducted. A total of 124 questionnaires was sent to producers. Some 42 producers returned the questionnaire. They had an annual cost of $55.54 to prevent contracting pseudorabies infection. The distance to infected farms did not have any bearing on the costs to prevent pseudorabies infection.

Other livestock producers surrounding infected swine herds were contacted. A total of 21 producers indicated no extra costs were incurred to prevent pseudorabies infection.

Table X and XI show the state and federal government costs of $101,721 and $17,546 respectively. Labor costs accounted for the major portion of the costs.
The pilot project to eradicate pseudorabies from Marshall County, Iowa is an attempt to determine the technical feasibility of pseudorabies (PR) eradication while investigating the incentives producers will have to participate in such a program along with the costs they must bear. The combining of epidemiological and economic studies in this pilot project can help assess the feasibility of a national eradication program.

The economic analysis to be carried out in Marshall County has seven objectives:

1. To determine the government costs of administering a pseudorabies eradication program.
2. To determine the direct costs to a producer of eradicating pseudorabies from a herd by following one of three specific plans.
3. To evaluate the probability of success of particular plans and the length of time required for eradication. Actions that must be repeated several times before successful eradication will affect not only the expected costs of eradication but also the risk faced by the producer.
4. To determine the benefits that accrue to an individual producer from not having pseudorabies in his herd. These benefits must be evaluated in light of the nature of the infection; whether it be clinical or subclinical, and whether vaccination is used or not.
5. To determine the costs to a producer of keeping pseudorabies out of his herd assuming the disease is prevalent in his area.
6. To determine the optimal strategy for a producer to follow and the associated profits given different assumptions about the prevalence of pseudorabies in his area.
7. To estimate the changes in producer net income from eradicating pseudorabies from Marshall County and maintaining a disease free area.

Project Design

The information necessary to evaluate the cost-benefit tradeoffs outlined above is being obtained by surveying producers in Marshall County. Thirty nine seropositive herds and twenty five negative herds are included in the survey. The producers in Marshall County were all personally interviewed in the summer of 1984 and again in the spring and summer of 1985. The survey form consists of five parts. Part I provides a description of the operation as to type, size, and facilities. The second part attempts to measure the costs of maintaining a pseudorabies negative herd. Direct costs of an outbreak are summarized in section three. The cost of the various cleanup programs are documented in the fourth section. The plans followed in Marshall County include a depopulation-repopulation pro-
gram, a test and removal program and a progeny segregation program. The final part of the survey collects production records during periods when the disease is present and when it is not.

The data collected through October 1985 do not give complete answers to the questions outlined above but do give some indication of the types of costs and benefits involved. This data will be discussed in the next sections as it relates to the goals of the analysis.

**Characteristics of Sample Herds**

The majority of the herds surveyed are farrow to finish producers. There are no clear differences in the clean and infected herds as to type of operation as evidenced by the data in Table I.

<table>
<thead>
<tr>
<th>Type of Operation</th>
<th>PR Negative</th>
<th>PR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedstock</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Farrow-to-Finish</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>Feeder Pig Producer</td>
<td>1 (FF/FPP = 2)</td>
<td>2 (FF/FPP = 3)</td>
</tr>
<tr>
<td>Feeder Pig Finisher</td>
<td>2 (FF/FPF = 1)</td>
<td>3 (FF/FPF = 6)</td>
</tr>
</tbody>
</table>

The average size of infected herds was about 50% larger than uninfected herds when measured by total number of pigs on the premises and over twice as large when measured by the size of the sow herd. The data in Table II supports the earlier report by Gustafson that pseudorabies seems to be more prevalent in larger herds.

<table>
<thead>
<tr>
<th></th>
<th>25 PRV Negative Herds</th>
<th>39 PRV Positive Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boars</td>
<td>3.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Gilts</td>
<td>24.9</td>
<td>33.1</td>
</tr>
<tr>
<td>Sows</td>
<td>44.3</td>
<td>111.2</td>
</tr>
<tr>
<td>Growers/Finishers</td>
<td>308.9</td>
<td>722.3</td>
</tr>
<tr>
<td>Suckling Pigs</td>
<td>92.6</td>
<td>237.4</td>
</tr>
<tr>
<td>Purchased Feeders</td>
<td>44.0</td>
<td>48.3</td>
</tr>
<tr>
<td>Total Herd Average</td>
<td>464.0</td>
<td>1018.7</td>
</tr>
</tbody>
</table>

There seems to be a correlation between the number of breeding stock purchased per year and pseudorabies infection. The clean herds in the sample purchased no female breeding stock. There did seem to be a higher turnover rate for boars in negative herds as evidenced by the data in Table III.
Table III
AVERAGE NUMBER OF SWINE PURCHASED PER YEAR PER HERD

<table>
<thead>
<tr>
<th></th>
<th>PR Negative</th>
<th>PR Postive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boars</td>
<td>3.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Gilts/Sows</td>
<td>0.0</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Factors Affecting Disease Transmission

In order to investigate the factors that might help to control the spread of pseudorabies a number of questions were asked concerning management practices. The results are summarized in Tables IV through VI.

Table IV
TESTING OF PURCHASED BREEDING STOCK FOR PR AT THE TIME OF PURCHASE

<table>
<thead>
<tr>
<th></th>
<th>PR Negative</th>
<th>PR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always</td>
<td>17.4%</td>
<td>19.4%</td>
</tr>
<tr>
<td>Usually</td>
<td>13.0%</td>
<td>13.9%</td>
</tr>
<tr>
<td>Never</td>
<td>69.6%</td>
<td>66.7%</td>
</tr>
</tbody>
</table>

Table V
ISOLATION AND OBSERVATION OF PURCHASED SWINE BEFORE COMMINGLING

<table>
<thead>
<tr>
<th></th>
<th>PR Negative</th>
<th>PR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always</td>
<td>56.0%</td>
<td>59.0%</td>
</tr>
<tr>
<td>Usually</td>
<td>24.0%</td>
<td>17.9%</td>
</tr>
<tr>
<td>Never</td>
<td>20.0%</td>
<td>23.1%</td>
</tr>
</tbody>
</table>

Surprisingly, there seemed to be little difference in the amount of prepurchase testing done by positive and negative herds. Of some concern was the fact that over 60% of the producers do not test purchased swine before adding them to the herd. About 20% of the producers do not isolate purchased swine before commingling in the herd. While these practices could help reduce the chances of contracting the virus there was not clear evidence of differences in practice between positive and negative herds.

Most of the positive herds in the sample vaccinated against pseudorabies to prevent clinical outbreaks. About half of the negative herds vaccinated as a precautionary measure. Many producers cited fear of neighborhood spread as a reason for vaccination in negative herds.

Table VI
PRESENTLY VACCINATING AGAINST PR

<table>
<thead>
<tr>
<th></th>
<th>PR Negative</th>
<th>PR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>11 (44%)</td>
<td>34 (85%)</td>
</tr>
<tr>
<td>No</td>
<td>14 (56%)</td>
<td>5 (15%)</td>
</tr>
</tbody>
</table>
Other Diseases in Sample Herds

There has been considerable discussion and speculation about possible subclinical effects of pseudorabies in reducing overall herd health. Questions were asked about herd medical histories, in particular, which diseases had confirmed diagnosed symptoms from January 1982 through August 1985. The results are summarized in Table VII.

Table VII
DIAGNOSED CASES OF DISEASE
FROM JANUARY, 1982 TO AUGUST, 1985

<table>
<thead>
<tr>
<th>Disease</th>
<th>25 PR Negative Herds</th>
<th>39 PR Positive Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Parvo virus</td>
<td>5 20.0%</td>
<td>5 12.8%</td>
</tr>
<tr>
<td>TGE</td>
<td>4 16.0%</td>
<td>15 38.5%</td>
</tr>
<tr>
<td>Haemophilus pleuropneu.</td>
<td>1 4.0%</td>
<td>9 23.1%</td>
</tr>
<tr>
<td>Mycoplasmal pneumonia</td>
<td>1 4.0%</td>
<td>7 17.9%</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>4 16.0%</td>
<td>1 2.6%</td>
</tr>
<tr>
<td>Dysentery</td>
<td>1 4.0%</td>
<td>1 2.6%</td>
</tr>
<tr>
<td>Salmonella</td>
<td>1 4.0%</td>
<td>5 12.8%</td>
</tr>
<tr>
<td>Other</td>
<td>3 12.0%</td>
<td>9 23.1%</td>
</tr>
<tr>
<td>a. Rhinitis</td>
<td>3 12.0%</td>
<td>4 10.3%</td>
</tr>
<tr>
<td>b. Campylobacter</td>
<td>0</td>
<td>2 5.1%</td>
</tr>
<tr>
<td>c. Neonatal scour</td>
<td>0</td>
<td>3 7.7%</td>
</tr>
</tbody>
</table>

There are clearly more clinical cases of TGE, mycoplasmal pneumonia and *Haemophilus pleuropneumonia* in the herds associated with pseudorabies infections. This difference might be associated with management practices, or subclinical effects of PR. More research will be needed to explain these findings.

Overall Herd Health

As a measure of overall herd health producers were asked about live births and pigs weaned per litter in the presence of pseudorabies. As indicated in Table VIII there was no difference between positive and negative herds in the Marshall County sample.

Table VIII
REPORTED LIVE PIGS AT BIRTH AND WEANING

<table>
<thead>
<tr>
<th>Measure</th>
<th>PR Negative</th>
<th>PR Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Births/Litter</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Pigs Weaned/Litter</td>
<td>8.3</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Costs of Pseudorabies

While data on the subclinical cost of pseudorabies are not yet conclusive, some discussion of the cost of clinical outbreaks can be undertaken. Of the thirty nine positive herds in Marshall County sixteen (41%) have experi-
enced a clinical outbreak at some time. This clearly points out the fact that clinical disease data does not adequately measure the extent of PR infection in an area.

Table IX
PRV POSITIVE HERDS: CLINICAL VERSUS SUBCLINICAL DISEASE

Clinical signs observed in 16 herds = 41.0%
No clinical signs observed in 23 herds = 59.0%

The actual physical losses sustained by herds during clinical outbreaks are summarized in Table X. These losses are losses above normal for herds experiencing these symptoms.

The actual cost of these losses to the producer will depend on a variety of assumptions and have not been summarized to date. The methods used to value these physical losses are discussed in a later section.

Table X
PHYSICAL LOSSES INCURRED DURING CLINICAL OUTBREAKS

Average Swine Losses

<table>
<thead>
<tr>
<th>Losses</th>
<th>Number</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abortions</td>
<td>6.1</td>
<td>$37.30</td>
</tr>
<tr>
<td>2. Embryo resorptions</td>
<td>8.9</td>
<td>249.25</td>
</tr>
<tr>
<td>3. Stillbirths/mummies</td>
<td>17.6</td>
<td>14.67</td>
</tr>
<tr>
<td>4. Deaths in suckling pigs</td>
<td>107.1</td>
<td>33.93</td>
</tr>
<tr>
<td>5. Deaths in growers/finishers</td>
<td>0.6</td>
<td>65.00</td>
</tr>
<tr>
<td>6. Deaths in breeders</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

The costs of controlling clinical outbreaks through vaccination and other measures are contained in Table XI. The actual direct costs of $400.15 are of course very small compared with the much larger losses resulting from animal deaths.

Table XI
Average Cost of Controlling Clinical Outbreaks

<table>
<thead>
<tr>
<th>Measures</th>
<th>Number</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Veterinarian</td>
<td></td>
<td>$37.30</td>
</tr>
<tr>
<td>2. PR vaccine</td>
<td>199.4 doses</td>
<td>249.25</td>
</tr>
<tr>
<td>3. Diagnostic lab</td>
<td></td>
<td>14.67</td>
</tr>
<tr>
<td>4. Drugs</td>
<td></td>
<td>33.93</td>
</tr>
<tr>
<td>5. Labor</td>
<td>13 hours</td>
<td>65.00</td>
</tr>
</tbody>
</table>

The costs of eradication on a per herd basis are presented in Table XII. These are costs for all herds, not just those which have been able to eradicate pseudorabies. These are costs on a cumulative basis and are not
annualized in any fashion. The program costs are primarily for vaccine and serology. The total program cost of well over $2,000 per herd is large compared to producer cost in the range of $400–$600 per herd. These costs do not include the overhead and administrative costs of the program. These costs will be summarized in the final report on the Iowa pilot project.

The primary producer cost is in selling off breeding stock at less than in herd value. The costs of segregation facilities have been relatively low as producers have generally been able to use existing facilities.

**Table XII**

**PR ERADICATION COSTS THROUGH AUGUST, 1985 ON 39 POSITIVE HERDS**

A. Costs borne by program on a per herd basis

1. Vaccine 1262.9 doses @ $1.25/dose = $ 1578.63

2. Serology
   a. 183.6 blood samples = 394.70
   b. 5.5 veterinary cells = 88.00
   $ 2,061.33

B. Costs borne by producers on a per herd basis

1. PRV positive breeders culled
   a. boars 0.9
   b. direct loss/boar $178.57
   c. performance time foregone 13 months
   d. gilts and sows 22.6
   e. direct loss/female $ 72.50
   f. litters foregone 2.2

2. Replacement breeders
   a. boars 1.2
   b. cost/boar $425.13
   c. gilts and sows 6.7
   d. cost/female $155.63

3. Cost of segregation
   a. labor 18.9 hours
   b. materials and maintenance $131.08

4. Disinfection and clean-up
   a. labor 55.0 hours
   b. materials $90.70

5. Transportation $8.99

**Valuing the Effects of a Pseudorabies Outbreak**

This section will summarize the methods that will be used to determine the economic losses to producers from a pseudorabies outbreak. In addition to the direct veterinary and vaccination costs associated with an outbreak there are costs associated with lost animals. Producers losses are best
measured by the lost returns from not being able to market a product. When animals die or there are selling restrictions the producer loses revenue from the sale of the animal but still incurs many of the expenses of production. For example, when a baby pig dies the producer loses the opportunity to market that animal at slaughter weight. The revenue he loses is the market price of the animal at market weight. This amount is not all lost however since he also does not incur the costs of feeding the animal. The extent of these costs depends on the assumption made about the production program. For example, if the producer is a feeder pig producer then the value of the baby pigs might be determined as using the following procedure.

i) Determine revenue foregone as the price per head of feeder pigs adjusted for typical death loss.

ii) Determine foregone costs (or costs not incurred) to include
   a) Feed for the pig but not for the sow during lactation if other pigs are still nursing.
   b) Health costs not incurred such as vaccinations that are made at a later date.
   c) Fuel, repairs and utility costs are not reduced by their full amount since many of these costs will not fall when just a few pigs die. These costs however are much less if many pigs die.
   d) Labor and interest cost is determined in a manner similar to fuel, repairs and utilities.

iii) The loss from losing the baby pig would be the revenue in (i) minus the costs in (ii).

This procedure assumes that a producers welfare can be measured by his returns above his variable costs. This measure is called quasirent and is given by

$$R = TR - TVC$$

when R is rent, TR is total revenue and TVC is total variable cost. If there are buying and selling restrictions the losses are determined by comparing rent in the two cases of sales without restrictions and sales with restrictions.

Summary
The data presented in this paper are preliminary and are meant to provide an indication of the type of results that will follow from further analysis. The costs of cleanup per herd have been relatively low given the good success rate in eradication. The actual benefit cost tradeoffs will depend in a large measure on the cost of cleaning up the remaining herds in the county and maintenance costs of keeping the area pseudorabies free.

Literature Cited
VIRUS CONCENTRATION PROCEDURES FOR ASSAY OF RUN-OFF WATER FROM FARMS WITH PSEUDORABIES INFECTED SWINE

Therese Brown, Merwin Frey, Clayton Kelling and Alex Hogg
Department of Veterinary Science, University of Nebraska-Lincoln

The first two sets of experiments were performed to test the virus concentration capabilities of a molecular filtration system that appeared to have advantages over previously used systems of virus recovery from water samples. Identical amounts of non-purified virus suspension (medium from infected cell cultures) were added in one experiment to 9 aliquots of sterile distilled water and in a later experiment to 24 aliquots of water from natural sources. The latter samples, which contained various amounts of particulate matter, were prepared using water from streams or lakes.

With all 9 of the samples of virus in distilled water there was a titer in the concentrated sample indicative of a greater amount of virus than that contained in the starting sample. This was attributed to a breaking up of viral aggregates with the concentration procedures, which included dilution in particle free water and subsequent sonication. There was also good, but relatively much lower, percentage recovery of the virus added to the 24 water samples from natural sources. The lower recovery rates (average 63%) were thought to be due to adsorption of virus to particulate matter.

With evidence that the methods used would concentrate pseudorabies virus without apparent loss of viral infectivity, samples of rain induced run-off water were collected from 10 farms having swine acutely infected with pseudorabies virus. At each farm two samples were collected simultaneously, utilizing an ISCO Model 1640 automatic water sampler placed in a drainage area or running stream within or just downstream from the swine lots. The sampler was automatically activated when run-off occurred during a rainstorm, collecting samples of approximately 6 liters.

At the laboratory bovine serum albumin was added to the sample, which was then sonicated, prefiltered, and run through the molecular filtration system. The concentrated samples were assayed for virus by passage in cell cultures and visualization by electron microscopy.

Pseudorabies virus was not isolated from or visualized microscopically in any of the surface water samples collected at the infected farms. It was concluded from these results that run-off water from farms with pseudorabies infected swine probably is not a major potential source of infection to herds located downstream.
Many of you attended the LCI meeting in which I gave a report on the progress of the pilot projects in the original five states plus Ohio. That talk was very well received. I accepted many compliments on the content of the talk and in fact USDA's Animal and Plant Health Inspection Service incorporated my remarks into a USDA press release. The reason I make mention of that presentation is that at the end of the talk I made two recommendations. One was that we begin to evaluate the pilot projects this fall even though the conclusion is not anticipated until October of 1986. The other recommendation was that we allow enough time and enough different alternatives to producers to eradicate the disease from their herds and from areas with the least amount of cost and suffering to those people involved.

I'd like to spend some time this afternoon talking about the first recommendation: that is that we begin to evaluate the pilot projects even before their anticipated completion. The first question that might legitimately be asked is, "why do a preliminary or a premature evaluation?" This question is especially relevant in consideration of the person and organization to whom it's posed. National Pork Producers Council has often rebuked others for basing policy on sketchy preliminary data. Therefore the question is one worth answering.

Let me begin answering that question by saying that we have a certain amount of momentum going with the pilot projects which we should give maximum effort to continuing. In addition all the projects are showing success, both in producer cooperation and the ability of those particular techniques to rid pseudorabies from a herd and from an area. There is no doubt that from the first attempt to design a national eradication program until the time that program is initiated that some period of time, probably years, will have elapsed. This has to do with the lengthy democratic process to achieve the federal support for a relatively large federal program. Of course you are aware of the federal budgetary process which includes USDA's building a budget, OMB taking that information and constructing a new budget and finally congressional overhaul of that budget. Each one of these steps takes a significant amount of time and in
total could result in a loss of much of the progress made during the pilot projects.

The other answer I give to the question of preliminary data analysis has to do with the nature of the results of the pilot projects. My concerns in releasing or evaluating preliminary data most often has to do with comparison of significant results. In the case of the pilot projects I see the results as being more societal and incremental. This is the case as opposed to being a situation where all the hard data must be collected before any analysis can take place. What I mean by societal is that I see the pilot projects being successful because we got good producer cooperation and support in almost all of the pilot project areas and any breakdown in cooperation had more to do with some of the regulatory personalities involved than anything else. What I mean by incremental, if that’s the right word, is that we can continually evaluate the progress and the success as the projects continue. It seems to me that we’re not in a situation where all the data must be collected before any analysis can take place. We are certainly in a position now to see which particular techniques and approaches work in different areas of concentration and different levels of infection.

Now I have explained why I made that recommendation to initiate some type of assessment of the projects but now you might ask, “Where do we go from here?” NPPC and LCI joined forces to begin to answer that question. It’s been decided to host a national PRV symposium based on the theme; “following up on the pilot projects—what next?” The symposium will be held in Peoria, Illinois at the Continental Regency Hotel on January 20 and 21, 1986. The symposium will be sponsored by USDA in cooperation with NPPC, LCI, USAHA, AAVLD, AASP, AVMA, NASR, NFPMA and AFBF.

No updates on the pilot projects will be provided at the meeting. There will not be any presentations of numbers and figures about what’s happened in the projects. Instead we are looking towards more of a philosophical discussion along the lines of what we learned in pilot projects. We anticipate a representative from each of the pilot project states to give their outline of what a national program should involve based on their retrospective analysis of the work in their pilot project program. I am hopeful that the individuals following me on this program today can give you a hint of this kind of discussion. I know I have talked with each of these people and have heard them go on in detailed discussion about what we have learned from the pilot projects and more specifically how they would approach the disease in a particular herd differently than they might have three years ago.

During the program of the symposium we will also have someone talk about the European experience. They did some things right over there but they did others all wrong and I think that we should make sure that we
benefit from their approach. We also will be looking briefly at the tests and testing procedures and how those would fit into any national program. Another discussion will center on the role of the vaccine in future programs. Of course a major consideration will be a report of the economic analysis in each of the pilot projects and especially the economic analysis taking place at Carroll Foods. Fortunately these economic studies will be concluded by the end of this year so that we will have hard final data to analyze in these cases. This information will be extremely valuable in any decision in the future of PRV programs in the U.S. Finally on that first day program we are considering a discussion of the feeder pig regulations which have been either enacted or are being considered in different states. We need to discuss how those regulations affect the national effort.

This second day is very important to any national policy decision on what the industry decides to do with this disease. The second day of the symposium will involve a commission hearing. Producers representing NPPC, LCI, AASP, NASR, NFPMA and AFBF and the Pork Action Group will be asked to sit on a commission hearing testimony from groups and individuals on their perceptions on the future course of action relative to PRV. We will be asking for these people requesting time on the program to make a statement to identify the alternative that they are supporting. The alternatives may be eradication, control, vaccination, or doing nothing.

Following the conclusion of this symposium the commission will meet again to assimilate all the information that has been presented and to develop some national suggested approach for addressing the PRV question. Of course, any recommendation developed by this commission would have to be considered by each of the individual group policy making bodies before any recommendation is made to USDA. However, it is important to have some policy structured in time for the USDA budget making process for the fiscal year 1988. This process translates into having a recommendation to USDA sometime in March of 1986 or soon thereafter.

Of course, the question will soon be asked about fiscal year 1987. We have been assured that continuation funds are incorporated in the USDA budget so that the work in the project areas can continue at least on a monitoring basis.

If the industry is to move forward in addressing this disease problem which has caused such a great emotional response in the past it will be very important to have maximum national support of the vast majority of individuals and organizations affected by the disease. We think that whatever program is developed from this PRV symposium in January will be solid, tested and credible.

We look forward to your attendance at that meeting to help us answer the question; "PRV—Where do we go from here?"

NATIONAL PSEUDORABIES CONTROL BOARD

This is a voluntary industry program sanctioned by the states. The effect
of status conferred by the Board is dependent on willingness of states to accept the status and permit movement of swine based on the status conferred by the Board.

**PURPOSE**—Purpose of the Board is to consider and act on applications for status of areas/states as low-prevalence pseudorabies areas as outlined in Criteria for Certifying Low-Prevalence PRV Areas.

**MEMBERSHIP**—The Board will be made up of two representatives each from the United States Animal Health Association, Livestock Conservation Institute and the National Pork Producers Council.

**PROCEDURES**—Headquarters of the Board will be the offices of the Livestock Conservation Institute. Those seeking recognition of status by the Board should submit 7 copies of an application for recognition of status to the offices of the Board documenting the following:

1. Authorities for carrying out the procedure as outlined in the criteria.
2. Any evidence available indicating the prevalence of PRV in the state/area under consideration.
3. Details of the surveillance, quarantine, herd cleanup and other procedures to be carried out in meeting the criteria for the status sought.
4. The status being sought.
5. Boundaries of the area under consideration.
6. Programs to control movements of swine into the area under consideration.
HEALTH IMPLICATIONS OF RAW MILK

Stanley L. Diesch, DVM, MPH*1
St. Paul, MN

In the United States the dairy industry began to industrialize in the 1800s. Mass production and distribution with poor sanitation and handling procedures, and disease in cows led to widespread human disease outbreaks.

From 1900 until the end of World War II milkborne diseases common in that period were typhoid fever, scarlet fever, septic sore throat, diphtheria, tuberculosis, shigellosis, and milk sickness. After milk was pasteurized, the outbreaks decreased dramatically.

In the early 1900s contaminated milk problems resulted in two public health movements:

1. the certified milk movement promoted sanitation in all phases of milk production and marketing under the oversite of the Medical Milk Commission. This group denounced pasteurized milk as causing nutritional deficiencies, allowing the marketing of sterilized filth, and destroyed the natural flavor of milk.

2. the pasteurized milk movement denounced the sale of raw milk (certified and uncertified) as unsafe despite sanitary precautions.

Despite no substantiated scientific evidence that milk pasteurization has an adverse effect on human nutrition and health, the sale of raw milk (certified and uncertified) has persisted. The volume of sale has increased in recent years due to the increased promotion of raw milk as health food.

Raw (unpasteurized) milk may be certified or uncertified. Certified raw milk is produced under standards and methods established by the American Association of Medical Milk Commissions, Inc.1 It began in 1893 as the Medical Milk Commission of Essex County, New Jersey. This milk now is produced by a few large dairies and sold in states where it is allowed. Uncertified raw milk is usually produced in small volumes by individual dairy-farmers and sold on the farm or by home delivery in areas permitting the practice.

In the United States pasteurization was first stressed nationally by the U.S. Public Health Service in its 1927 Milk Ordinance Code. Pasteurized milk is heat treated for specified time and temperature combinations and designed to kill all micro-organisms that transmit disease to humans. Exposing milk at or above 145°F (63°C) for 30 minutes, to 161°F (72°C) for 15 seconds or 191°F (89°C) for one second kills the human pathogenic organisms.2 Contemporary milkborne diseases of importance in the U.S.A. are salmonellosis, campylobacteriosis, staphylococcal intoxication,

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brucellosis, yersiniosis, listeriosis, *Escherichia coli*, and streptococcal infection.

**Nutritional Values in Raw and Pasteurized Milk**

Advocates of raw milk indicate that pasteurization of milk causes changes detrimental to human health. Pasteurization affects six milk constituents with known nutritional benefits. Three vitamins for which milk is a minor source (thiamine, B₁₂, and C) and also calcium, protein, and fat. Vitamins are reduced about 10%. About 6% of the calcium becomes insoluble, about 1% of milk protein is coagulated, and there is a slight disaggregation of fat globules. This has no effect on the bioavailability of these three nutrients. Raw milk is said by some to contain certain undefined and undetected health promoters such as an anti stiffness factor, valuable in treating arthritis. These claims have not been substantiated by documented research.³

**Diseases Associated with Raw (Unpasteurized) Milk**

Numerous disease outbreaks have been documented and associated with certified and uncertified raw milk.

**Salmonellosis and Raw Milk**

In 1981, 46 cases of human *Salmonella dublin* infection were reported in California; in 1982, 70 cases. In both years 24% of the patients used certified raw milk. In 1983, 123 cases of *S. dublin* were identified; of 99 persons, 44% reported using certified raw milk. Twenty-six percent of those hospitalized died. It was reported that the relative risk of illness from *S. dublin* for certified milk consumers was 158.0 times greater in California than for those who do not drink any form of raw milk. Many were also stressed with other diseases and conditions. *S. dublin* is a strongly host-adapted serotype found in cattle.⁴ *S. typhimurium* has been associated with consumers of raw milk in five outbreaks in Arizona, three in Oregon, and six in Quebec.

The epidemiology of human and animal infections of *S. dublin* in the U.S.A. was reviewed from the early 1960s to 1982. The authors reported that a high proportion of human infections were associated with drinking raw milk. Animal isolates also show a high resistance to commonly used antibiotics.⁵

As low as one *Salmonella* organism/100g of food has resulted in human infection and disease.⁶ Infective doses for humans were reported in six of 11 outbreaks studied. The actual doses ingested were calculated to be \(<10^3\) organisms. The outbreaks with higher doses involved very high rates of attack and short periods of incubation.⁷ *Salmonella* contamination of raw milk may be intermittent and low grade and contamination may not be distributed uniformly in a day’s production. In February 1981, (one day survey) the Food and Drug Administration cultured certified raw milk and found 20% of the containers (4 of 20 half gallon cartons) contaminated with *Salmonellae*. These were produced and bottled by one dairy on the same day.⁸
Twenty outbreaks of milk borne salmonellosis affecting more than 518 people were reported in England and Wales during 1983–84. In 19 outbreaks, raw milk was implicated, in the other contaminated pasteurized milk. The implementation of 1983 legislation in Scotland requiring heat treatment of cow’s milk for sale to the public has reduced the incidence of milkborne infection from previous years. In England and Wales, without legislative control, outbreaks continue to occur.9

**Campylobacteriosis and Raw Milk**

Fourteen (61%) of 23 *Campylobacter* outbreaks reported to the Centers for Disease Control from 1980–1982 were traced to consumption of raw milk. In the U.S.A. campylobacteriosis outbreaks associated with drinking unpasteurized milk have recently been reported in Arizona, California, Colorado, Georgia, Kansas, Maine, Oregon, and Pennsylvania.3

In Minnesota following a one day field trip to a Minnesota farm, 22 of 49 third grade students and three of 21 adult chaperones developed Campylobacter enteritis. Illness was associated with unpasteurized milk consumption during a picnic lunch and participation in hand milking cows. Epidemiologic findings implicated unpasteurized milk as the source. The dairy farm, inspected twice yearly by the Minnesota Department of Agriculture, was a modern, well-run operation of 180 cows.10

**Toxoplasmosis and Raw Goat’s Milk**

In California, ten of 24 members of an extended family had serologic evidence of acute *Toxoplasma* infection. All ten seropositive persons had recently consumed raw goat’s milk from the family herd. The other 14 serologically negative members had not consumed milk. Raw goat’s milk consumption showed the strongest association with toxoplasmosis.11

**Unidentified Enteritis Associated with Raw Milk**

During July 1984, the Minnesota Health Department (MHD) investigated an outbreak of chronic diarrhea among 122 residents of Brainerd, Minnesota. Consumption of raw milk from a single dairy farm was associated with illness. Illness was characterized by acute onset of diarrhea with marked urgency, a lack of systemic symptoms and failure to respond to multiple antimicrobial agents. Multiple attempts to identify any etiologic agents with cultural and immunologic studies for over 80 different organisms was unsuccessful. During April 1985, 115 of the original 122 (94%) case patients were re-interviewed to learn of their clinical status. Only 19 (24%) of the patients had recovered. The duration of illness among those not yet recovered ranged from 274 to 443 days (median 353). For those recovered, duration of illness ranged from 29 to 416 days (median 205 days). Since the Brainerd investigation, outbreaks and sporadic cases of a similar illness of unknown etiology have been recognized among raw milk drinkers in at least seven states.12,13

Minnesota law clearly defines requirements for commercial sale of only pasteurized milk. However, the law allows for purchase of unpasteurized
milk and cream for personal use by the consumer at the place or farm where milk is produced. While all pasteurized milk sold must be labeled, a consumer can purchase raw milk directly from a dairy farmer in the consumer's own container. Based on three surveys 5–10% of Minnesotans, many of whom are rural, drink raw milk.

**Raw Milk and Tuberculosis**

In 1985 an outbreak of tuberculosis has occurred in cattle in New Mexico in four infected herds in which 337 reactors have been identified. Additional testing of herds and traceback studies are being conducted. Over 900 employees and families have been drinking raw milk from the dairies. A number of these have been placed on INH preventative treatment. The practice of dairy farms supplying raw milk to their employees and families constitutes a disease risk factor throughout the United States.

**Disease Outbreaks Associated with Pasteurized Milk Products**

Milk, being an ideal medium for maintenance or growth of some infectious disease agents despite pasteurization, has not been totally protected from failures in the system and other sources of post pasteurization contamination.

**Human Outbreak of Yersinia**

In June and July 1982, a large outbreak (estimated at several thousand) of *Yersinia enterocolitica* caused by an unusual serotype occurred in Tennessee, Arkansas, and Mississippi. Epidemiologic evidence associated cases with drinking pasteurized milk from a specific plant. Inspection of the plant and culturing available raw and pasteurized milk did not reveal the source or mechanism of contamination or incorrect pasteurization procedures. The ability of *Y. enterocolitica* to grow in milk at refrigeration temperatures makes pasteurized milk a possible vehicle for virulent *Yersinia*. Proper pasteurization temperature is effective in killing *Yersinia*. Widespread presence of *Yersinia enterocolitica* in nature and farm animals provide opportunities for virulent strains to enter the food chain including milk.

**Listeriosis Outbreak in Massachusetts**

Between June 30 and August 30, 1983, 49 people in Massachusetts acquired listeriosis. Seven cases occurred in fetuses or infants and 42 in immunosuppressed adults; 14 patients (29%) died. Following epidemiologic studies the illness was strongly associated with the drinking of a specific brand of pasteurized whole or 2 per cent milk. Multiple serotypes of *L. monocytogenes* were isolated from raw milk obtained from a group of farms supplying milk and where listeriosis in dairy cows occurred at the time of outbreak. At the plant processing the milk, inspection revealed no evidence of improper pasteurization. This outbreak has raised questions about the ability of pasteurization to eradicate a large inoculum of *L. monocytogenes* from contaminated raw milk.
The bacteria has the ability to exist as an intracellular parasite. This could have increased the ability to survive pasteurization. Before pasteurization, most homogenized milk undergoes a centrifugal filtering process called clarification which removes parasites. In Massachusetts the whole milk was passed through a milk filter rather than clarified, so that leukocytes were not removed. In this situation pasteurization may have only been partly effective. Researchers have reported the D-value at 71.7°C was 0.9 seconds. *L. monocytogenes* would not survive the pasteurization process of milk. This would indicate that pasteurization temperature is very adequate to destroy the bacteria.

**Listeriosis Associated with Cheese**

In California, in 1985, Mexican-style pasteurized cheeses produced in a plant were found to contain *L. monocytogenes*. The FDA warned residents of 13 states not to eat this brand of cheese. Sixty human deaths were linked to this cheese. An audit of records by the State Food and Agriculture Department revealed the amount of unpasteurized milk received by the plant far surpassed the company's pasteurization capacity. California officials indicated that they believed that Listeria found in Cacique cheese “came from unpasteurized cheese.”

**Illinois Salmonella Outbreak**

The salmonella epidemic which struck Illinois and five additional states in March 1985, resulted in 18,000 reported cases. On September 14, 1985, a final report of an investigation by a 21-member Task Force was released. The final report determined that a strain of *Salmonella typhimurium* using (antibiograms and/or plasmid analyses) caused at least three outbreaks over 8 months. The Task Force concluded that the epidemic strain was likely to have persisted within the plant environment during that time or intermittent re-introduction from a persistent focus outside the plant. The Task Force also concluded that a cross connection was likely to have been responsible for the continued intermittent contamination of pasteurized milk by the outbreak strain.

The report offered eight recommendations for assuring milk safety in the future:

1. Consistent compliance with Pasteurized Milk Ordinancy (PMO), including all of its technical phases.
2. Maintenance of updated, accurate engineering drawings and flow charts kept current by both regulatory officials and processors.
3. Development and implementation of meaningful container coding.
4. Maintenance of complete production records and retention of these records for at least one year.
5. Education and training of key dairy and regulatory employees in processing and in public health aspects of dairy science.
6. Certification of pasteurizer operators.
8. Evaluation of critical control points with emphasis on pasteurization and blending systems, related piping, and methods for reclaiming milk."

This epidemic resulted as a breakdown in process, not in the pasteurization itself.20

Scientific Support for Pasteurization

Most infectious diseases epidemiologists and public health professionals consider the scientific evidence against raw milk and for pasteurized milk to be irrefutable. During the past several years several national health organizations, the United States Animal Health Association, the American Veterinary Medical Association (AVMA), the American Academy of Pediatrics, the National Association of State Public Health Veterinarians, and the Conference of State and Territorial Epidemiologists have adopted policy statements which recommend that milk and milk products for human consumption be pasteurized.

The AVMA had recommended in the states where sale is legal that raw milk carry a warning label: “Not pasteurized and may contain organisms that cause human disease.”

In January 1985, Arizona, which permits sale of raw milk required a warning label: “Raw milk: not pasteurized and may contain organisms injurious to your health.” In 1985 New Mexico has also adapted labeling of raw milk as recommended by the AVMA.

Food and Drug Administration Public Hearing

On April 10, 1984, Dr. Sidney Wolfe of the Public Citizen Health Research Group filed a petition to ban all domestic sales of raw (i.e. unpasteurized) milk and raw milk products.

On October 11–12, 1984, the Department of Health and Human Services, Food and Drug Administration, held a public hearing in Washington, D.C., to receive information on whether milk and milk products for human consumption should be pasteurized. During the hearings chaired by Commissioner Frank Young (FDA), 17 individuals representing themselves or organizations spoke against regulations requiring pasteurization and 13 spoke for regulations requiring pasteurization. Included in the 13 were representatives from the National Conference on Interstate Milk Shipments, National Milk Producers Federation, and Mid-America Dairymen.

At this hearing the speakers addressed two issues: (1) whether the consumption of raw milk, including certified raw milk and milk products, is of public concern; and (2) if the answer to the first question is yes, whether requiring of pasteurization of raw milk, including certified raw milk and raw milk products, is the most reasonable regulatory option.

AVMA Support of Pasteurization

At this hearing I represented the American Veterinary Medical Association in testimony. The statement had been prepared by the AVMA
Council on Public Health and Regulatory Veterinary Medicine. In summary the resolution stated:

1. Because apparently healthy cows and goats can shed microorganisms in their milk that cause human disease (pathogens); and
2. Because milk handlers and the environment can introduce pathogenic organisms into raw milk (including certified raw milk)
3. Only pasteurized milk and milk products should be sold for human consumption.

Veterinary medical scientists and others interested in animal agriculture have made great advances in controlling diseases of milk-producing animals, but problems still exist. Producers are unable to assure that raw milk and raw milk products are free of pathogenic organisms such as campylobacter, salmonella, and streptococci for the following reasons:

1. The indoor and outdoor environments of milk-producing animals (barns, feeding areas, pastures, and animal waste holding and storage facilities) are ready sources of pathogenic organisms.
2. Contaminated feed and water can introduce and maintain the carrier state in milk-producing animals.
3. Milk producing animals can carry and shed many pathogenic organisms without showing clinical signs.
4. In general, drug therapy is uneconomical and is not effective in eliminating some pathogenic organisms (especially campylobacter and salmonella species) from milk-producing carrier animals.

Although brucellosis and tuberculosis are considered controlled in milk-producing animals, occasional outbreaks still occur and the movement of animals from farm to farm, and state to state complicates and reduces effectiveness of the control programs. Other diseases such as leptospirosis, Q fever, salmonellosis, *Escherichia coli* bacillosis, streptococcosis, listeriosis, and toxoplasmosis can be subclinical in cattle and goats. Periodic testing or examination of the animals gives no assurance that the animals will not contract diseases between testing periods and produce contaminated milk.

Commissioner Frank Young, Chair of the hearing, specifically requested that I provide additional information concerning the quantitative detection of *Salmonella dublin* in infected cattle herds. The statement presented was that:

“At present we lack sufficiently sensitive or specific cultural or immunological techniques to accurately detect either *S. dublin* infected herds or individual animals. Particularly with culture results, a positive result is meaningful, but little confidence can be placed on negative results.”

This information further substantiates that despite advances in veterinary medicine, assurance cannot be given that milk cows are free of
potential human pathogens such as *S. dublin*, even if test results are negative.

**FDA’s Decision**

The FDA’s action was as follows: that at the present time standardized milk and milk products shipped in interstate commerce with the exception of “certified raw milk” must be pasteurized.

The consumption of raw milk and raw milk products presents a public health problem. Evidence submitted to the hearing record establishes an increased risk to individuals consuming certified raw milk, particularly if there are attendant medical factors that lower the consumer’s resistance to infectious disease. Furthermore, there were no significant health benefits identified from the consumption of certified raw milk. Thus, a public health problem exists.

They felt that additional federal regulation of raw milk would be neither effective nor appropriate. The raw milk problem arises only because a minority of states have declined to ban raw milk and that the FDA would be involved only where the interstate commerce is significantly affected.21,22

According to information available, 31 states prohibit the intrastate sale of unpasteurized milk and milk products. In the 19 states allowing sale of raw milk there is no inhibition by federal law against prohibiting intrastate sale of raw milk or raw milk products.

During the twentieth century, milk pasteurization has been a major contributor to the dramatic reduction in the United States of the occurrence of milk-borne diseases such as bovine brucellosis and tuberculosis. Pasteurization provides a strong safeguard against the risk of drinking milk contaminated with bacteria and viruses harmful to human health. Pasteurization subjects milk to high temperatures during processing for a brief but sufficient time to destroy any disease producing organisms without substantially affecting the flavor or nutrient quality of the natural milk. Subsequently the milk is rapidly cooled, packaged in sealed containers, and refrigerated to preclude the re-introduction and growth of pathogenic micro-organisms.

Today, in the United States properly pasteurized milk and milk products, properly handled and stored after pasteurization, are rarely implicated in disease transmission. The American public accepts milk and milk products as highly nutritious foods. Consumers expect milk to be free of pathogens and safe to drink.

The October 1984 Food and Drug Administrative hearings further substantiated that a public health problem exists as a result of drinking raw milk, but refused to take action on interstate or intrastate sale of raw milk. If raw milk consumption continues to increase, a potential damage to the dairy industry exists. The health professions and dairy industries in each of the approximately 19 states should combine their efforts to assure
the public that milk marketed and consumed is properly pasteurized in order to provide a wholesome, safe human food.

REFERENCES


HEALTH IMPLICATIONS OF RAW MILK


REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND ENVIRONMENTAL QUALITY

Chairman: A. J. Roth, Richmond, Virginia

Vice Chairman: Robert H. Singer, Winchester, Kentucky

Members: F. J. Alderink, MD; F. M. Applehans, TN; A. W. Bialey, OK; R. P. Crawford, TX; S. L. Diesch, MN; C. R. Dorn, OH; J. A. Farrar, FL; S. L. Hendricks, MN; W. E. Jennings, TX; J. C. Leighty, MD; E. L. Mennings, VA; W. R. Miller, MD; R. L. Parker, SC; J. E. Pearson, IA; J. C. Prucha, MD; D. F. Schwindaman, MD; T. P. Siburt, VA; C. D. Stumpff, KS; M. E. Potter, GA; R. E. Lowe, MD.

The Committee on Public Health and Environmental Quality met at 1:30 p.m., Wednesday, October 30, 1985, as scheduled. A total of 11 members and 28 guests were in attendance.

The Subcommittee on topics for discussion at next year’s annual meeting were reported by Dr. Ed Menning. The subcommittee suggested 8 topics. These will be evaluated and those 4 or 5 topics that can be reported will constitute our next year’s agenda.

The subcommittee on Future Challenges and Concerns in Public Health identified 17 potentially problematic topics which should be resolvable goals for future committee members. The committee report is attached. (A-1)

Dr. C. D. Stumpff presented an excellent update on program activities and the number of tuberculosis cases in cattle this year. He stressed that Mexico should be encouraged to institute an effective tuberculosis control and eradication program. He reported that there were 30 herds of cattle found to be infected with bovine tuberculosis during the past year.

Dr. Robert Singer presented a paper on health hazards related to ethylene and dimethyl thiocarbamate pesticides. He described the hazards via the breakdown of these products to man and animals. Further, he described cases, involving horses and those people residing on the farm exposed to the pesticide drift and residues.

Dr. Jack Leighty discussed methods involved in certifying swine as trichina free. He related that the Food Safety and Inspection Service has been involved in three somewhat different aspects of trichinosis control. These involve a new regulation on control of trichina in processed pork products, testing to certify that pork is trichina free, and irradiation.

He reported that in April, 1985, a group of experts from the Netherlands, Canada, and the United States evaluated the developmental work that his agency had done up to that point on the trichinosis enzyme immunoassay. They concluded that our work is scientifically sound and that the assay is a sensitive, reliable and reproducible method of examining swine sera for the presence of antitrichina antibodies.

On July 22nd the Food and Drug Administration approved a petition submitted by Radiation Technology Inc. asking that irradiation of fresh...
pork at levels of 30 to 1000 kilorads be permitted. The approval related to this method is considered by FDA at present to be a food additive. Presently, the question of labeling irradiated pork has not been decided. The labeling of other irradiated foods is now under consideration at FDA. The USDA and FDA policies will be coordinated.

The problems are basically twofold: the increase or decrease in risk of transmission from livestock and poultry to people of bacterial, rickettsial, and chlamydial, infections; and the compromise of human therapy due to infections caused by "superbugs" transmitted to people from livestock or poultry fed subtherapeutic levels of antibiotics. Approximately 47% of the antibiotics used in the U.S. are fed subtherapeutically to livestock and poultry.

He selected 16 major zoonotic outbreaks of bacterial diseases for consideration. His illustrations are attached to this report. (A-2)

The Health Implications of Raw Milk was presented by Dr. S. L. Diesch. This report was presented to you by Dr. Diesch this morning, thus I will not report on his subject.

Dr. Morris Potter from CDC discussed very briefly and answered questions concerning the task force report on a salmonellosis outbreak in Illinois.

The meeting was adjourned at 4:15 p.m.
4. The practice of leaving animals dead of toxic residues to the environment as a result of renderers refusal to remove such animals because of legal implications.

5. Agricultural chemicals and ground water.

6. Biomedical research and animal rights advocates.

7. Food safety problems stemming from increased utilization of convenience foods and fast-food outlets.

8. The development and implementation of new technologies for meat and poultry inspection.

9. A traceback system for food animals.

10. A joint effort between the Public Health and Environmental Quality Committee and the Salmonella Committee for the purpose of formulating a comprehensive nationwide proposal to be presented to USDA for the reduction of salmonelosis in both (food) animals and man.

11. Antibiotic resistance in pathogenic bacteria and the therapeutic and subtherapeutic use of antibiotics in food animals, animal feeds, and human beings.

12. The chain of transmission of foodborne pathogens between man and animal.

13. Development of quality control programs in food animal husbandry.

14. Development of increased concern for public health among all parts of the veterinary medical profession, particularly among educators.

15. Better methods of detection of foodborne pathogens, including viruses.

16. Improved methodology for detection of foodborne viruses to determine the contributions of these viruses to diseases of human beings.

17. Develop methods of prevention of foodborne viral diseases of man and animals.

Respectfully submitted
W. R. Miller
Subcommittee Chairman

ZOONOTIC DISEASE OUTBREAKS
ASSOCIATION WITH PLASMID MEDIATED ANTIBIOTIC RESISTANT BACTERIA

ZOONOSIS: Salmonellosis associated with ground beef—Central United States.

ETIOLOGICAL AGENT
IDENTIFICATION: Salmonella newport

ANTIBIOTIC RESISTANCE: Tetracycline, penicillin, carbenicillin, ampicillin, streptomycin, sulfonamide
METHOD OF TRANSMISSION: Ingestion of ground beef contaminated with *S. newport*

OUTBREAK OF HUMAN DISEASE

LOCATION: South Dakota, Minnesota, Iowa, North Dakota
TIME: December 1982–May 1983
REPORTED CASES: 17 meat associated & 1 nosocomial case
REPORTED DEATHS: 1 nosocomial case
COMPLICATIONS: 12 of the patients were taking penicillin or ampicillin for other medical reasons prior to onset

ASSOCIATED ANIMALS

SPECIES: Beef cattle were the source of the contaminated meat

CLINICAL DISEASE: None in the beef cattle. Clinical salmonellosis was diagnosed in a neighboring dairy herd one month earlier

ANTIBIOTIC USE: Therapeutic use in dairy calves; subtherapeutic feeding of chlortetracycline in the beef herd

ZOONOSIS: Salmonellosis associated with imported beef—Pennsylvania and New Jersey

ETIOLOGICAL AGENT

IDENTIFICATION: *Salmonella typhimurium*, *S. newport* and *S. chester*

ANTIBIOTIC RESISTANCE: All were sensitive to all antibiotics tested; the *S. typhimurium* had no plasmids

METHOD OF TRANSMISSION: Consumption of inadequately cooked or recontaminated imported beef

OUTBREAK OF HUMAN DISEASE

LOCATION: Pennsylvania and New Jersey
TIME: June–August, 1981
REPORTED CASES: 89: 47 in New Jersey and 42 in Philadelphia
REPORTED DEATHS: None
COMPLICATIONS:

ASSOCIATED ANIMALS

SPECIES: Beef cattle

CLINICAL DISEASE: None reported

ANTIBIOTIC USE: None reported

ZOONOSIS: Salmonellosis associated with turkey giblets—Maine

ETIOLOGICAL AGENT

IDENTIFICATION: *Salmonella enteritidis*
ANTIBIOTIC RESISTANCE: Multiply sensitive

METHOD OF TRANSMISSION: Ingestion of inadequately cooked giblet gravy

OUTBREAK OF HUMAN DISEASE

LOCATION: Maine
TIME: November 25–27, 1982
REPORTED CASES: 112 culture confirmed cases
REPORTED DEATHS: None
COMPLICATIONS: Oxidized refrigerated giblets had cooked appearance when they were actually raw

ASSOCIATED ANIMALS

SPECIES: Turkeys
CLINICAL DISEASE: None
ANTIBIOTIC USE: None reported

ZOONOSIS: Salmonellosis associated with milk—Illinois

ETIOLOGICAL AGENT

IDENTIFICATION: *Salmonella typhimurium*

ANTIBIOTIC RESISTANCE: Penicillin, ampicillin, tetracycline, carbenicillin, streptomycin, sulfisoxazole. Sensitive to chloramphenicol, trimethaprim-sulfamethoxazole, cepalothin, gentamycin, kanamycin, naladixic acid, and trimethaprim

METHOD OF TRANSMISSION: Consumption of 2% lowfat milk processed in Melrose Park, IL March 20 and March 30, 1985

OUTBREAK OF HUMAN DISEASE

LOCATION: 94% of cases in Illinois. Cases in Indiana, Iowa and Michigan where same milk sold and in Wisconsin, Minnesota and Florida in persons returning to their states
TIME: March 22–Mid April, 1985
REPORTED CASES: Over 18,000 cases reported with over 14,500 laboratory confirmed
REPORTED DEATHS: 4 associated cases; 1 of these reported as primary cause
COMPLICATIONS: One patient who died received penicillin injection for abscessed tooth 2 days before onset of salmonellosis

ASSOCIATED ANIMALS

SPECIES: Suspect dairy cattle; liquid egg transport under investigation
CLINICAL DISEASE: None associated
ANTIBIOTIC USE: Not yet reported
5
ZOONOSIS: Salmonellosis associated with inadequately pasteurized milk—Kentucky

ETIOLOGICAL AGENT
IDENTIFICATION: *Salmonella typhimurium*

ANTIBIOTIC RESISTANCE: Isolates from milk and patients had the same plasmids, Tetracycline resistant

METHOD OF TRANSMISSION: Consumption of inadequately pasteurized milk

OUTBREAK OF HUMAN DISEASE
LOCATION: Western Kentucky
TIME: March 28–May 2, 1984
REPORTED CASES: 16
REPORTED DEATHS: None
COMPLICATIONS: Milk was vat pasteurized but temperatures were too low

ASSOCIATED ANIMALS
SPECIES: Dairy cattle

CLINICAL DISEASE: Recurrent diarrhea reported but not diagnosed

ANTIBIOTIC USE: Therapeutic use

6
ZOONOSIS: Salmonellosis associated with raw milk—Montana

ETIOLOGICAL AGENT
IDENTIFICATION: *Salmonella typhimurium*

ANTIBIOTIC RESISTANCE: Ampicillin, tetracycline, streptomycin, kanamycin, sulfonamides and cephalothin

METHOD OF TRANSMISSION: Consumption of raw milk from a local dairy

OUTBREAK OF HUMAN DISEASE
LOCATION: Western Montana
REPORTED CASES: 105
REPORTED DEATHS: None
COMPLICATIONS: Contamination of milk appears to have been from personnel during processing

ASSOCIATED ANIMALS
SPECIES: Dairy cattle

CLINICAL DISEASE: None. No evidence of the infection was found at the dairy

ANTIBIOTIC USE: None
ZOOONOSIS: Salmonellosis associated with eating cheese—Eastern Canada

Etiological Agent
Identification: *Salmonella typhimurium*, phage type 10
Antibiotic Resistance: Not reported
Method of Transmission: Consumption of cheese from a plant on Prince Edward Island

Outbreak of Human Disease
Location: Prince Edward Island, Newfoundland, New Brunswick and Ontario, Canada
Time: First half of 1984
Reported Cases: Several hundred
Reported Deaths: None
Complications: Several types and labels of cheeses involved

Associated Animals
Species: Dairy cattle
Clinical Disease: None
Antibiotic Use: None reported

ZOOONOSIS: Salmonellosis associated with dairy calves—Connecticut

Etiological Agent
Identification: *Salmonella heidelberg*
Antibiotic Resistance: Tetracycline, sulfamethoxazole, chloramphenicol
Method of Transmission: Direct contact from dairy calves to farmers and a pregnant handler; *in utero* or by contact to infant son, then to 2 other infants by nursery attendants

Outbreak of Human Disease
Location: Northern Connecticut
Time: August–September 1976
Reported Cases: 5; 2 adults by animal contact, 3 infants by human-human transmission
Reported Deaths: None
Complications: Human-to-human transmission was in infants in a hospital nursery. This appears to be a very vulnerable population

Associated Animals
Species: 70 dairy calves under 1 week old bought at several auctions
Clinical Disease: Diarrhea; one calf died
ANTIBIOTIC USE: Therapeutic in the calves. Three strains isolated from calves and one from the farmer had resistance to the 3 antibiotics listed plus to neomycin, streptomycin, kanamycin

ZOO NOSIS: Salmonellosis associated with dairy calves—England

ETIOLOGICAL AGENT

IDENTIFICATION: Salmonella typhimurium phage type 29

ANTIBIOTIC RESISTANCE: Multiply resistant: streptomycin, sulfonamides, tetracycline, ampicillin, neomycin, kanamycin, furazolidone chloramphenicol

METHOD OF TRANSMISSION: Contact with infected calves distributed throughout England from a single dealer

OUTBREAK OF HUMAN DISEASE

LOCATION: Widespread in England
TIME: 1963–1969 with peak in 1965
REPORTED CASES: 555 reported in 1965
REPORTED DEATHS: 6

COMPLICATIONS: Appears to begin with a tetracycline-resistant strain which acquired multiple resistance as spread in calves

ASSOCIATED ANIMALS

SPECIES: Dairy calves

CLINICAL DISEASE: Severe, with over 50% mortality in many infected herds. During 1964–1966, a total of 1657 cases were reported in calves

ANTIBIOTIC USE: Therapeutic. Every available antibiotic was used

ZOO NOSIS: Ground beef associated hemorrhagic colitis

ETIOLOGICAL AGENT

IDENTIFICATION: Eschericia coli 0157:H7

ANTIBIOTIC RESISTANCE: Multiply resistant. Tetracycline resistant

METHOD OF TRANSMISSION: Undercooked ground beef in specialty hamburgers served by a fast food chain

OUTBREAK OF HUMAN DISEASE

LOCATION: Oregon and Michigan
TIME: February–March and May–June, 1982
REPORTED CASES: 47
REPORTED DEATHS: None

COMPLICATIONS: Tetracycline or erythromycin therapy not effective
ASSOCIATED ANIMALS

SPECIES: Beef possibly contaminated by handlers. *E. coli* 0157:H7 has not been identified from animals in the U.S.

CLINICAL DISEASE: None

ANTIBIOTIC USE: None reported

ZOOINOSIS: Campylobacteriosis associated with dressed chicken—Washington

ETIOLOGICAL AGENT

IDENTIFICATION: *Campylobacter jejuni*

ANTIBIOTIC RESISTANCE: Resistance to 1 or more of ampicillin, streptomycin or tetracycline in 42% of human, 45% of retail chicken and 23% of processor poultry isolates.

METHOD OF TRANSMISSION: Handling or consumption of chicken

OUTBREAK OF HUMAN DISEASE

LOCATION: Seattle—King County, Washington

TIME: February 1982—September 1983

REPORTED CASES: 105 poultry associated cases

REPORTED DEATHS: None

COMPLICATIONS:

ASSOCIATED ANIMALS

SPECIES: Broiler chickens

CLINICAL DISEASE: None

ANTIBIOTIC USE: Considered used subtherapeutically in source poultry

ZOOINOSIS: Campylobacteriosis jejuni associated with raw milk—Pennsylvania

ETIOLOGICAL AGENT

IDENTIFICATION: *Campylobacter jejuni*

ANTIBIOTIC RESISTANCE: Not reported

METHOD OF TRANSMISSION: Consumption of raw milk

OUTBREAK OF HUMAN DISEASE

LOCATION: 2 contact farms in central Pennsylvania

TIME: May, 1983

REPORTED CASES: 31/63 and 26/45 visitors to the 2 farms

REPORTED DEATHS: None

COMPLICATIONS:

ASSOCIATED ANIMALS
SPECIES: Dairy cattle
CLINICAL DISEASE: None
ANTIBIOTIC USE: None reported

ZOONOSIS: Campylobacteriosis associated with improperly pasteurized milk—England

ETIOLOGICAL AGENT
IDENTIFICATION: Campylobacter jejuni
ANTIBIOTIC RESISTANCE: Not reported
METHOD OF TRANSMISSION: Consumption of milk in a school following failure of the pasteurizer

OUTBREAK OF HUMAN DISEASE
LOCATION: Bedfordshire, England
TIME: March, 1979
REPORTED CASES: About 3500
REPORTED DEATHS: None

ASSOCIATED ANIMALS
SPECIES: Dairy cattle
CLINICAL DISEASE: None
ANTIBIOTIC USE: None reported

ZOONOSIS: Listeriosis associated with fresh cheese—California

ETIOLOGICAL AGENT
IDENTIFICATION: Listeria monocytogenes, most serotype 46
ANTIBIOTIC RESISTANCE: Not reported
METHOD OF TRANSMISSION: Consumption of Mexican style fresh cheese

OUTBREAK OF HUMAN DISEASE
LOCATION: Los Angeles and Orange Counties, California
TIME: January–June, 1985
REPORTED CASES: 86, of which 58 were mother-infant pairs
REPORTED DEATHS: 29, of which 8 were neonatal, 13 stillbirths

ASSOCIATED ANIMALS
SPECIES: Dairy cattle
CLINICAL DISEASE: None
ANTIBIOTIC USE: None reported
ZOO NOSIS: Leptospirosis associated with swine—Alabama

ETIOLOGICAL AGENT

IDENTIFICATION: *Leptospira interrogans* *pomona*

ANTIBIOTIC RESISTANCE: Not reported

METHOD OF TRANSMISSION: Waterborne contact through swimming in a creek contaminated by dead swine

OUTBREAK OF HUMAN DISEASE

LOCATION: Geneva, Alabama

TIME: July, 1950

REPORTED CASES: About 50 clinical cases

REPORTED DEATHS: None

COMPLICATIONS:

ASSOCIATED ANIMALS

SPECIES: Swine

CLINICAL DISEASE: None

ANTIBIOTIC USE: None reported

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ZOO NOSIS: Erysipeloid associated with swine—Pennsylvania

ETIOLOGICAL AGENT

IDENTIFICATION: *Erysipelothrix rhusiopathiae*

ANTIBIOTIC RESISTANCE: Not reported

METHOD OF TRANSMISSION: Direct contact from tissues of infected slaughter hogs to abraded or broken skin of packing house personnel

OUTBREAK OF HUMAN DISEASE

LOCATION: Philadelphia

TIME: 1936–1938

REPORTED CASES: 400, all male packing plant personnel

REPORTED DEATHS: None

COMPLICATIONS:

ASSOCIATED ANIMALS

SPECIES: Swine

CLINICAL DISEASE: Various—mostly chronically infected, also incubatory carriers

ANTIBIOTIC USE: None
IMMUNE COMPLEX-LIKE DISEASE IN TWO GROUPS OF PERSONS FOLLOWING A BOOSTER DOSE OF RABIES HUMAN DIPLOID CELL VACCINE


From the College of Veterinary Medicine (Dreesen, J. Brown, Kemp) and University Health Services (W. J. Brown), The University of Georgia, Athens 30602, and the U.S. Public Health Service, Centers for Disease Control, Atlanta, GA 30333 (Fishbein, Bernard, Parker, Yager).

Summary

Following a routine 0.1 ml booster dose of Merieux rabies human diploid cell vaccine (M-HDCV) administered intradermally (ID), 23 (10.2%) of 226 persons at the College of Veterinary Medicine, The University of Georgia, Athens had signs and symptoms compatible with an immune complex-like disease. Manifestation of signs began 3–13 days after injection and consisted of urticaria (78.3%), macular rash (65.2%), angio edema (39.1%), and arthralgia (17.4%) which lasted 1–5 days. Similar reactions have been reported using both M-HDCV and Wyeth HDCV administered either ID or intramuscularly. The number of adverse reactions following the routine administration of M-HDCV booster doses is the largest seen to date.

To determine if this phenomenon was an uncommon occurrence, a clinical trial was designed in which 38 persons received a single Connaught Laboratories Ltd, Toronto, human diploid cell vaccine (C-HDCV) booster administered 0.1 ml ID, while a second group of 40 persons received a single booster dose of M-HDCV similarly administered. As before, 10% (4 of 40) of the persons who received M-HDCV had adverse systemic reactions while none of those receiving C-HDCV had reactions that could be classified as immune complex-like. It is concluded that M-HDCV may cause adverse reactions in up to 10% of persons receiving a booster immunization, thus individuals who dispense or receive the vaccine in this manner should be aware of this problem.

Introduction

Worldwide, well over two million doses of rabies human diploid cell vaccine (HDCV) have been used for rabies pre- and post-exposure since the vaccine became generally available in Europe in 1974. Since 1980, when HDCV was first licensed in the United States, over 30,000 persons at high risk of exposure to rabies have received pre-exposure HDCV by a 3-dose immunization series, administered intramuscularly (IM) or intradermally (ID).1 Although local reactions have been fairly common following the

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malaise from 3–13 days after the booster dose. Clinical impressions were obtained by personal interviews, visits to physicians, and questionnaires completed by the patient. All 23 cases were interviewed by either 1 or 2 physicians. Fifteen to 23 days after the booster, blood samples for rabies serology were obtained from 161 of the 226 persons including all 23 cases.

Signs and symptoms meeting the case definitions occurred in 23 (10.2%) of 226 persons from 3–13 days (median, 10 days) after the booster; they lasted 1–5 days (median, 3 days). The 23 persons who reacted had one or more of the following: a generalized urticaria (18 cases, 78.3%); macular rash primarily affecting the trunk, legs and arms (15 cases, 65.2%); angioedema of the face, especially the lips, the hands and feet (9 cases, 39.1%); and arthralgia of wrists, knees, and ankles (4 cases, 17.4%). One person reported transitory dyspnea in addition to edema and urticaria, and another complained of pyrexia and malaise as well as signs of edema, urticaria, and arthralgia. These signs were rather dramatic and quite discomforting, 18 of the 23 lost from one to three days of work time or class attendance; however, none of the patients or attending physicians considered the disease to be severe or life threatening. Sixteen persons took H₁ receptor-blocking antihistamines (e.g., chlorpheniramine or diphenhydramine), including two who also received epinephrine. Five received corticosteroid therapy. The remaining two received no therapy. All 23 persons recovered with no sequelae.

Based on questionnaire responses, each case was individually matched to a nonreactor (control) for comparing RFFIT titers. A control was considered as a person who had received the HDCV booster but showed no evidence of adverse systemic reactions within a two week period following the booster. The cases and controls were matched based on three principal variables: sex, age (± 3 years), and occupational group (i.e., faculty, student by class, staff, etc.). As far as possible, cases and controls were also matched for history of previous rabies immunizations, local reactions following the booster, known allergies (presence/absence), and “feeling under stress.” The Merieux HDCV contains the Wistar PM strain of rabies virus grown in human diploid cells. It also contains human serum albumin and neomycin and is inactivated with beta-propiolactone. Following this 1984 episode, skin tests of cases and non-cases were conducted (discussed later) and studies on serum specimens were also done at the United States Food and Drug Administration. These studies indicated the possibility that a beta-propiolactone-altered human serum albumin fraction in the vaccine was sensitizing receptors in certain individuals and triggering immunoglobulin E production resulting in the allergic reactions (H. Baer, Allergic Products Branch, FDA: personal communication).

1985: 4 Systemic Reactions in 40 Persons

Again, in the early Spring of 1985, another 99 persons at the College of Veterinary Medicine were due to receive a two-year booster dose of rabies HDCV as recommended. A clinical trial was designed to aid in determining whether or not the altered human serum albumin fraction of the
primary series, systemic allergic reactions are extremely rare following primary pre-exposure immunization.\textsuperscript{2-5}

The Advisory Committee on Immunization Practices (ACIP) of the U.S. Public Health Services currently recommends that persons receiving the 3-dose primary series of HDCV either be given a single booster dose every two years or have rabies neutralizing antibody titer determined and be given a booster dose only if the titer is inadequate.\textsuperscript{1} Within the past three years, the Centers for Disease Control (CDC) in Atlanta, Georgia, have received reports of approximately 125 cases of allergic reactions clinically compatible with Type III immune complex-like disease as a sequela to a single booster immunization. Systemic reactions have followed both IM and ID administration of the HDCV booster and have occurred with both the Merieux Laboratories product (the only one currently available in the United States) and the Wyeth Laboratories HDCV which was licensed for use in the U.S. until February 1985.\textsuperscript{5,6}

This report concerns a total of 27 cases of systemic immune complex-like disease (22\% of all reported cases). These cases occurred in two groups of individuals in 1984 and 1985 at the College of Veterinary Medicine, The University of Georgia, Athens, Georgia, following a single 0.1 ml ID booster of Merieux rabies human diploid cell vaccine (M-HDCV): The vaccine was administered to 266 persons approximately two years after they had received a primary three-dose ID series of M-HDCV.

**The Problem**

**1984: 23 Systemic Reactions in 226 Persons**

In February 1984, 226 persons received a single 0.1 ml booster dose of M-HDCV (Merieux Laboratories lot X0791, antigenic value 4.4 IU/ml) injected ID into the lateral aspect of the upper deltoid. This was a routine vaccination given according to recommendations of the ACIP.\textsuperscript{1} Two years previously all 226 had received a primary pre-exposure series consisting of three ID doses, 0.1 ml each of M-HDCV given on days 0, 7, and 28. At the time this booster was administered, we were unaware of any reports of systemic, immune complex-like reactions occurring following use of rabies HDCV as a booster. Thus, this booster was given routinely without obtaining pre-booster blood specimens for serum rabies antibodies.

The mean age of the 226 persons was 27 years; 119 (52.6\%) were men and 107 (47.4\%) were women. Twenty were faculty members, 69 were members of the class of 1984, 83 were members of the class of 1985, 7 were graduate students, 35 were staff members, and 12 were from other groups on campus (miscellaneous classification).

A questionnaire was distributed to all available persons who had received the vaccine shortly after the first two cases of the immune complex-like disease were seen. A case was considered to be a reaction in a person who showed one or more of the following within two weeks of the HDCV booster: generalized or pruritic rash, urticaria, arthralgia, angioedema, and/or fever sometimes accompanied by nausea, vomiting, or
M-HDCV was indeed a factor in the adverse reactions. Recently, Con-
naught Laboratories, Ltd, Toronto, Canada, has developed a new, modified
HDCV (C-HDCV) that may be less reactogenic. The C-HDCV is produced
in a similar manner as is M-HDCV; however, the C-HDCV is subjected to
zonal-density centrifugation to remove the putative allergenic material,
the beta-propriolactone altered human serum albumin fraction. C-HDCV
has recently undergone testing in Canada and the United States, and
preliminary analysis shows it to be as immunogenic as M-HDCV when
used in a three-dose primary immunizing series (CDC unpublished data).

Of the 99 persons who were to receive boosters or have titers determined,
80 agreed to participate in the trial and 78 completed the full program. All
persons signed an informed consent as required by the University Human
Subjects Committee. These volunteers were divided into two equal groups
using a table of random numbers. Group I consisted of 38 persons (21
males, 17 females; mean age 32.4 years—range 24–54 years), all of whom
had received M-HDCV as a primary series two years previously. Twenty-
three were in the class of 1986, three in the class of 1987, and 12 were
faculty and staff. Each person received a single ID 0.1 ml dose of C-HDCV
(lot 1025-1, antigenic value 4.2 IU) in the upper deltoid. A 10–12 ml blood
specimen was obtained at the time of C-HDCV injection (day 0) and seven
and 28 days after injection. Blood serum was maintained at −70 C until
antibody was determined by the rapid fluorescent focus inhibition test
(RFFIT) at the Centers for Disease Control, Rabies Laboratory, Law-
renceville, Georgia. Group II consisted of 40 persons (23 males, 17 fe-
males; mean age 27.5 years—range 24–47). Thirty-one were in the class of
1986, one in the class of 1987, and eight were faculty and staff. Each of
these volunteers had also received a primary rabies M-HDCV series two
years previously. These 40 persons received M-HDCV (lot Y0423, an-
tigenic value 3.8 IU) in the same way as group I with blood obtained and
antibody determined as described above for group I persons.

Of the persons receiving M-HDCV, 10% (4 of 40) showed signs typical of
a systemic immune complex disease. This proportion was not significantly
different (Z = 0.03, p = 0.98) than the proportion (10.2%, 23 of 226)
obtained in 1984. None of the 38 persons who received the C-HDCV
showed signs compatible with the immune complex disease syndrome. The
difference between 0 and 10% is significant (Z = 2.00, p = 0.04).

In the 1985 episode, of the four persons who reacted, one had onset of
signs of the disease on day three after injection while the other three had
onset on day nine, the signs lasting two to eight days. Urticara and pruritis
were common to all four persons but the intensity was quite variable, from
wheels and welts of the face, extremities, trunk, and groin area to only
mild pruritic wheels on trunk. Two persons reported mild edema of the
eyelids. No one reported joint pain. All four were in the class of 1986, two
men and two women; three of the four lost either one or two days of class
time. All recovered with no sequelae.

RFFIT titers were logarithmically transformed and statistically anal-
alyzed. It was found that day 0 geometric mean titers (GMTs) of the two groups were not significantly different: group I 1:118, group II 1:166 (Figure I). On day 7 and day 28 the GMTs of group I (C-HDCV) were 1:574 and 1:910, respectively. These titers were significantly lower (p < 0.05) than the GMTs of 1:918 and 1:1683 for group II (M-HDCV) for the same time intervals.

Discussion of Results

Based on observed clinical cases, there appears to be a disproportionate number (22% of all reported cases in U.S.) and percentage (10% as compared to about 5% in U.S.) of adverse systemic reactions at the College of Veterinary Medicine, University of Georgia after a single 0.1 ml ID booster dose of M-HDCV.⁵ We have attempted to determine why this "Georgia Phenomenon" is occurring.

It is recognized that active surveillance will often identify undiagnosed or unrecognized disease. Although the clinical disease seen here was quite evident, it must be noted that a large number of persons (23 in 1984) had compatible signs of disease within a short time frame and, in 1985 in the designed clinical trial, we were aggressively searching for cases. In such instances, cases may be discovered that would otherwise be unrecognized, especially since most signs appear nine days or so after administration of the vaccine.

A few general observations can be presented from the cases of immune complex disease that have occurred at Georgia.

In the 1984 episode, males were at significantly greater risk than females (ratio 3.6:1, X² = 5.64, p = 0.02). These 226 people were arbitrarily divided into three groups and given the boosters on three separate days for convenience of administration. The subgroup (n = 99) who received the vaccine on the second day were at significantly higher risk (X² = 16.23, p = 0.0003) than those who received the booster dose on the other two days. Why males were a greater risk and why day of administration influenced adverse reactions is unclear and certainly suggests further study.

The titer range for the 23 cases in 1984 was 1:250 to > 1:7000 with a GMT of 1:1287. The titer range for the matched control group of 23 was 1:125 to 1:5400, GMT of 1:829. These titers were determined on serum obtained from 15–23 days after injection of the M-HDCV booster. A paired t-test showed there was no significant difference between the GMTs of the cases and controls. Thus, in the 1984 group, titer apparently had no influence on risk of adverse systemic reactions.

The GMT for the four persons who showed evidence of systemic allergic reactions in 1985 were compared to the 36 non-cases in the Merieux HDCV recipient group for days 0, 7, and 28. The GMTs for days 0, 7, and 28 for the four cases were 1:187, 1:820, and 1:2955. The GMTs for the non-cases for the same time intervals were 1:164, 1:915, and 1:1581. It was determined that there were no differences in GMTs between cases and non-cases when
comparisons were made for GMT at each interval. This corroborates the conclusions derived from comparisons of titers of matched cases and non-cases in the 1984 episode. It is concluded that titer does not influence risk of reaction.

It must be emphasized that all persons in both 1984 and 1985 exceeded the minimum recommended adequate titer of complete neutralization at the 1:5 dilution on the RFFIT. Studies are underway to determine if the statistically lower GMT noted for the Connaught recipients on days 7 and 28 has any biological significance (Figure 1).

Based on 1984 responses on questionnaires, there was no statistical difference between cases and non-cases for age, additional prior rabies vaccinations, local reactions following booster immunizations, known allergies of any kind, or "increased stress." No formal questionnaires were used in the 1985 clinical trial, but we determined that there was no difference in age and local reactions between cases and non-cases within the M-HDCV group or when cases were compared to the C-HDCV group participants.

Following the 1984 episode, immediate skin test (ST) reactivity to the immunizing agent was studied in six of the 23 cases and in a vaccinated control group.\textsuperscript{10} Comparison for reactivity was determined for these two groups of six persons for the same lot of M-HDCV used for boosters and a density gradient purified rabies PCEC (Behringwerke) that is free of the betapropriolactone-altered human serum albumin. The six cases were ST positive to the M-HDCV but not to the Behringwerke vaccine (BV). All in the non-symptomatic control group were ST negative for both vaccines. These findings, plus the unpublished, preliminary studies being conducted at FDA by Dr. H. Baer, and the clinical trial conducted by us in 1985, seem to indicate that the altered human serum albumin in the Merieux product is the factor triggering the reactivity manifested as an immune complex disease syndrome. This conclusion is further enforced by the conclusions arrived at from questionnaire response and serologic studies.

In summary, up to 10% of persons receiving rabies HDCV as currently produced by Institut Merieux, Lyon, France, as a booster dose according to current ACIP recommendations may show varying signs of a clinical immune complex-like hypersensitivity from two to 14 days following the injection. All persons administering or receiving the Merieux HDCV as boosters should be aware of the potential for a time delayed adverse reaction and appropriate therapy instituted as warranted by the severity of the reaction.
MEAN RABIES ANTIBODY TITERS IN TWO GROUPS OF PERSONS PRIOR TO AND FOLLOWING A SINGLE 0.1 ML BOOSTER DOSE OF TWO HUMAN DIPLOID CELL VACCINES ADMINISTERED INTRADERMALLY TWO YEARS AFTER A PRIMARY SERIES

CONNAUGHT HDCV (N=38)  MERIEUX HDCV (N=40)

RABIES NEUTRALIZING TITER

DAYS AFTER BOOSTER INOCULATION
REFERENCES


EVALUATION OF MERIEUX HUMAN
DIPLOID CELL RABIES VACCINE (M-HDCV):
A THREE YEAR STUDY

Dennis R. Howard
Deborah J. Briggs

SUMMARY

Human diploid cell rabies vaccine produced by Institute Merieux, Lyon, France was administered to approximately 200 volunteers. The volunteers were divided into four groups and all received primary immunization using 0.1 ml amounts intradermally in the deltoid area. Booster doses were given either intradermally or intramuscularly 1, 2, and 3 years post primary immunization. The antibody responses and reactions were studied for three years.

Introduction

The purpose of the study was to demonstrate the efficacy and safety of intradermal immunization using Merieux human diploid cell rabies vaccine (M-HDCV) for pre-exposure rabies prophylaxis in high risk populations.

After the introduction of duck embryo vaccine in 1957, the relative safety of the vaccine led to its use for pre-exposure prophylaxis in those with high risk of exposure to rabies — veterinarians, veterinary students, certain laboratory workers, animal handlers, foresters, spelunkers, and persons living in highly endemic areas.¹

Pre-exposure prophylaxis is generally recommended for the following reasons.
1) It provides protection from inapparent exposure to rabies,
2) It provides protection in those whose post-exposure therapy might be delayed,
3) A rapid anamnestic antibody response to additional doses of vaccine eliminates the need for rabies immune globulin and markedly reduces the number of doses needed should post-exposure treatment be necessary.²

In this study, the antibody responses and reactions demonstrated the safety and efficacy of intradermal pre-exposure immunization using M-HDCV.

Materials & Methods

Vaccine

M-HDCV was provided to Kansas State University Colleges of Veterinary Medicine. M-HDCV is produced by the Institute Merieux, Lyon, France and is a sterile, stable, freeze-dried suspension of rabies virus prepared from strain PM-1503-3M obtained from the Wistar Institute, Philadelphia, PA.
The virus is harvested from infected human diploid cell, MRC-5 strain, concentrated and inactivated by beta propiolactone. It is lympholized and packaged with a separate 1 ml vial of diluent. One ml of reconstituted vaccine contains less than 100 mg of human albumin, 150 mcg phenol red. The potency of M-HDCV is equal or greater than 2.5 international units of rabies antigen.

**Volunteers**

Subjects were selected from volunteers at the Kansas State College of Veterinary Medicine. Individual patient files were kept and included a consent form, reaction questionnaire, information report and results of testing for antibodies to rabies.

The volunteers were divided into four groups of between 20 and 80 each. Each of the volunteers had no previous rabies vaccination history. All participants were asked about current medications, medical problems and allergies. The answers were recorded on their information sheet.

**Study Design**

Vaccination schedule and route of administration is presented in Table I. Groups A1, A2, B1 and B2 received 0.1 cc M-HDCV intradermally (ID) in the left deltoid area by tuberculin syringe on days 0, 7 and 28. Booster injections were given to group A1 and A2 one, two, and three years following primary immunization either by the ID (Group A1) or intramuscular IM route (Group A2). Group B1 was given an ID booster and Group B2 was given a 1M booster two years after the primary series.

**Serum Collection and Antibody Determination**

Bloodsamples were drawn by peripheral venipuncture on days 0, 49, 90 and 365 on each of the volunteers regardless of the group they were assigned. Additional blood samples were collected from each group 10 days and thirty days after booster injections Table I.

Serum titers were determined by the rapid fluorescent focus inhibition test (RFFIT). The titer was expressed in international units IU per milliliter of serum. Geometric mean titers were calculated for each group of samples.

**RESULTS**

**Antibody Responses**

**Primary Immunization**

All individuals were tested negative for rabies antibody on day 0. Geometric mean titers on day 49 (21 days after the last dose of vaccine), 90 and 365 were 20.3 IU, 5.24 IU and 1.75 IU respectively. Adequate antibody titers were present in all subjects of each group one year after primary intradermal immunization.

**Booster Immunization**

Group A1 received a 0.1 cc ID booster injection and group A2 received a
A THREE YEAR STUDY

1.0 cc 1M booster injection 1, 2, and 3 years after primary immunization. Blood samples were obtained from each participant in each A group 10 and 30 days after booster immunization. Geometric mean titers were calculated and presented in Table 2.

Group B1 received a 0.1 cc lD booster injection and group B2 received a 1.0 cc 1M booster injection two years after primary immunization. Blood samples were obtained from each participant in each B group ten and thirty days after booster immunization. Geometric mean titers were calculated and are presented in Table 2.

Geometric mean titers of each group and individual titers of approximately 200 participants were greater than the minimum accepted level regardless of route of injection or booster schedule.

Adverse Reactions

Clinical reactions were recorded by a single individual from KSU Student Health Center and were based on history, physical examination and were done at the time of subsequent vaccination or blood collection.

Reactions are reported in tables 4-7 for each group. All reaction was considered minor except for two individuals in group B2 who developed a delayed type III hypersensitivity reaction on days 9 and 10. Titers from both individuals were 0.5 IU before booster injection.

Discussion

The results of this study suggest that M-HDCV is both safe and efficacious when used for pre-exposure prophylaxis. Geometric mean titers were above the minimum accepted level one year after primary immunization (2.28 IU for Group A1 and 1.8 IU for group B1 and 1.07 IU for group B2). World Health Organization suggests that titers of 0.5 IU to be adequate and the Advisory Committee on Immunization Practices (ACIP) suggests that complete neutralization at the 1:5 dilution by the RFFIT to be adequate. Of the approximately 200 participants of the study, no one failed to respond with an adequate titer 1 or 2 years after primary immunization. There appeared to be little difference in titers for participants in group A whether boosted by the 1M or 1D route Fig. 1. Several participants in each group had titers in excess of 70 IU.

Participants in group B were boosted two years after primary immunization and titers were not significantly different regardless of route of immunization either 1D (group B1) or 1M (group B2) Fig. 2.

Titers of Groups A1 and B1 were almost identical after two and three years. Both groups were boosted by the 1D route but group A1 received an additional booster dose at year one Fig. 3

Group A2 had a somewhat higher titer at year two and three than group B2 but almost identical titers at one year Fig. 4.

Regardless of booster injection route 1M or 1D, yearly or at two years, all participants had titers above the minimum accepted level. It appeared that an annual booster was not necessary and from the graphs the titers
would have been greater than the minimum accepted level even after two years.

The current ACIP recommendations are the persons working with live rabies virus in research laboratories or in vaccine production facilities have rabies antibody titers checked every six months and boosters. Only laboratory workers such as those doing rabies diagnostic tests, spelunkers and veterinarians, animal control and wildlife officers in areas where rabies in epizootic should have boosters every two years or have their serum tested for rabies antibody every two years and if the titer is inadequate, have a booster dose.4

The results from this study suggest M-HDCV to be safe and highly immunogenic when 0.1 ml doses are given for pre-exposure booster prophylaxis.

Approximately 1% of the participants reacted adversely with immune complex disease. The reactions were not life threatening and were readily treated with antihistamine therapy. This study does not agree with other literature suggesting that an “immune complex-like” illness may occur in up to 6% of persons receiving booster vaccines.5

**References**


Figure 1

RABIES - INTRADERMAL STUDY
KSU - Merieux

A THREE YEAR STUDY
RABIES-INTRADERMAL STUDY
KSU - Merieux

Figure 3
Figure 4

RABIES-INTRADERMAL STUDY
KSU - Merieux

Titer - IU

Month

1982
1983
1984
1985

F M A M J J A S O N D J F M

0 10 20 30 40 50 60 70
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Students</th>
<th>Immunization Schedule</th>
<th>Vaccine Dose</th>
<th>Route of Injection</th>
<th>Serum Samples for RFFIT</th>
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</thead>
<tbody>
<tr>
<td>A1</td>
<td>80</td>
<td>Day 0</td>
<td>0.1 ml I.D.</td>
<td>Days 0, 49, 90</td>
<td>Days 0, 49, 90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0.1 ml I.D.</td>
<td>Year 1, Year 1 &amp; Days 10, 30</td>
<td>Year 1, Year 1 &amp; Days 10, 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>0.1 ml I.D.</td>
<td>Year 2, Year 2 &amp; Days 10, 30</td>
<td>Year 2, Year 2 &amp; Days 10, 30</td>
</tr>
<tr>
<td></td>
<td>Year 1</td>
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<td></td>
<td>Year 3, Year 3 &amp; Days 10, 30</td>
<td>Year 3, Year 3 &amp; Days 10, 30</td>
</tr>
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</tr>
<tr>
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<td>0.1 ml I.D.</td>
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<td>Same as A1</td>
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<td>0.1 ml I.D.</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td>28</td>
<td>0.1 ml I.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year 1</td>
<td>1.0 ml I.M.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>1.0 ml I.M.</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>1.0 ml I.M.</td>
<td></td>
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</tr>
<tr>
<td>B1</td>
<td>80</td>
<td>Day 0</td>
<td>0.1 ml I.D.</td>
<td>Days 0, 49, 90</td>
<td>Days 0, 49, 90</td>
</tr>
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<td>0.1 ml I.D.</td>
<td>Year 1, Year 2, Year 2 &amp; Days 10, 30</td>
<td>Year 1, Year 2, Year 2 &amp; Days 10, 30</td>
</tr>
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<td>0.1 ml I.D.</td>
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<tr>
<td></td>
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<td>Year 3</td>
<td>Year 3</td>
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<tr>
<td></td>
<td>3</td>
<td>None</td>
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<td></td>
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</tr>
<tr>
<td>B2</td>
<td>20</td>
<td>Day 0</td>
<td>0.1 ml I.D.</td>
<td>Same as B1</td>
<td>Same as B1</td>
</tr>
<tr>
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<td>0.1 ml I.D.</td>
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<tr>
<td></td>
<td></td>
<td>28</td>
<td>0.1 ml I.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year 1</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.0 ml I.M.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>3</td>
<td>None</td>
<td></td>
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</tbody>
</table>
Table 2  Rabies Antibody Titers in Subjects Given Primary Pre-exposure Immunization with M-HDCV and Booster Immunization By The ID or IM Route on Year 1, 2 and 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>A 1</th>
<th>A 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ID Booster</td>
<td>IM Booster</td>
</tr>
<tr>
<td>1 year</td>
<td>2.28*</td>
<td>1.8</td>
</tr>
<tr>
<td>1 yr. 10 days</td>
<td>&gt;45.85</td>
<td>&gt;61.19</td>
</tr>
<tr>
<td>1 yr. 30 days</td>
<td>&gt;42.69</td>
<td>&gt;56.39</td>
</tr>
<tr>
<td>2 years</td>
<td>&gt;9.12</td>
<td>&gt;15.4</td>
</tr>
<tr>
<td>2 yr. 10 days</td>
<td>&gt;29.48</td>
<td>&gt;43.58</td>
</tr>
<tr>
<td>2 yr. 30 days</td>
<td>&gt;21.97</td>
<td>&gt;37.48</td>
</tr>
<tr>
<td>3 years</td>
<td>&gt;15.03</td>
<td>&gt;29.52</td>
</tr>
<tr>
<td>3 yr. 10 days</td>
<td>&gt;30.24</td>
<td>&gt;41.10</td>
</tr>
<tr>
<td>3 yr. 30 days</td>
<td>&gt;16.37</td>
<td>&gt;22.88</td>
</tr>
</tbody>
</table>

Titer expressed in International Units/ML.
Table 3  Rabies Antibody Titers In Subjects Given Primary Pre-exposure Immunization with M-HDCV and Booster Immunization By The ID Or IM Route On Year 2.

Group 2

<table>
<thead>
<tr>
<th></th>
<th>B1</th>
<th></th>
<th>B2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ID Booster</td>
<td>IM Booster</td>
<td></td>
</tr>
<tr>
<td>1 year</td>
<td>1.86*</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>1.03</td>
<td>0.78</td>
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</tr>
<tr>
<td>2 yr. 10 days</td>
<td>&gt;28.5</td>
<td>&gt;33.84</td>
<td></td>
</tr>
<tr>
<td>2 yr. 30 days</td>
<td>&gt;20.9</td>
<td>&gt;21.22</td>
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</tr>
<tr>
<td>3 years</td>
<td>&gt;13.22</td>
<td>&gt;10.34</td>
<td></td>
</tr>
</tbody>
</table>

* Titer expressed In International Unit/ML.
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Group A1 Primary Immunization</th>
<th>1 yr. Boost</th>
<th>2 yr. Boost</th>
<th>3 yr. Boost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redness at inj. site</td>
<td>47/74 (63%)</td>
<td>62/74 (83%)</td>
<td>51/73 (68%)</td>
<td>16/74 (22%)</td>
</tr>
<tr>
<td>Pain at inj. site</td>
<td>7/74 (9%)</td>
<td>15/74 (20%)</td>
<td>10/74 (13%)</td>
<td>12/74 (16%)</td>
</tr>
<tr>
<td>Swelling at site</td>
<td>18/74 (24%)</td>
<td>35/74 (47%)</td>
<td>25/74 (33%)</td>
<td>14/74 (19%)</td>
</tr>
<tr>
<td>Warmth at site</td>
<td>6/74 (8%)</td>
<td>15/74 (20%)</td>
<td>12/74 (16%)</td>
<td>10/74 (14%)</td>
</tr>
<tr>
<td>Itching at site</td>
<td>16/74 (21%)</td>
<td>29/74 (39%)</td>
<td>24/74 (32%)</td>
<td>15/74 (20%)</td>
</tr>
<tr>
<td>Itching generalized</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Fever</td>
<td>3/74 (4%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Muscle aches</td>
<td>2/74 (3%)</td>
<td>4/74 (5%)</td>
<td>5/73 (7%)</td>
<td>2/70 (3%)</td>
</tr>
<tr>
<td>Joint pains</td>
<td>2/74 (3%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Lymph local</td>
<td>12/74 (16%)</td>
<td>2/74 (3%)</td>
<td>5/74 (7%)</td>
<td>8/73 (11%)</td>
</tr>
<tr>
<td>Lymph syst.</td>
<td>2/74 (3%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Malaise</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>2/74 (3%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Shock</td>
<td>2/74 (3%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Headache</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Paining</td>
<td>2/74 (3%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>Rash</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
<td>1/74 (1%)</td>
</tr>
<tr>
<td>No reaction</td>
<td>16/74 (21%)</td>
<td>8/74 (10%)</td>
<td>22/74 (29%)</td>
<td>57/74 (77%)</td>
</tr>
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</table>
Table 5  Reactions following ID primary immunization and IM booster immunization.

<table>
<thead>
<tr>
<th>Group A2 Primary Immunization</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 28</th>
<th>1 Year Boost</th>
<th>2 Yr. Boost</th>
<th>3 Yr. Boost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>I.M. Booster I.M. Booster I.M. Booster</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness at inj. site</td>
<td>12/20 (60%)</td>
<td>17/20 (85%)</td>
<td>18/20 (90%)</td>
<td>2/20 (10%)</td>
<td>4/20 (20%)</td>
<td></td>
</tr>
<tr>
<td>Pain at inj. site</td>
<td>2/20 (10%)</td>
<td>6/20 (30%)</td>
<td>6/20 (30%)</td>
<td>2/20 (10%)</td>
<td>10/20 (50%)</td>
<td>10/20 (50%)</td>
</tr>
<tr>
<td>Swelling at site</td>
<td>3/20 (15%)</td>
<td>8/20 (40%)</td>
<td>11/20 (55%)</td>
<td>1/20 (5%)</td>
<td>4/20 (20%)</td>
<td>4/20 (20%)</td>
</tr>
<tr>
<td>Warmth at site</td>
<td>1/20 (5%)</td>
<td>3/20 (15%)</td>
<td>4/20 (20%)</td>
<td>1/20 (5%)</td>
<td>6/20 (30%)</td>
<td>3/20 (15%)</td>
</tr>
<tr>
<td>Itching at site</td>
<td>7/20 (35%)</td>
<td>9/20 (45%)</td>
<td>11/20 (55%)</td>
<td></td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
</tr>
<tr>
<td>Itching generalized</td>
<td>1/20 (5%)</td>
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<tr>
<td>Fever</td>
<td>1/20 (5%)</td>
<td>1/20 (5%)</td>
<td>2/20 (10%)</td>
<td>3/20 (15%)</td>
<td>3/20 (15%)</td>
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<tr>
<td>Muscle aches</td>
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<tr>
<td>Joint pain</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph local</td>
<td>2/20 (10%)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Lymph syst.</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Malaise</td>
<td></td>
<td></td>
<td></td>
<td>1/20 (5%)</td>
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<td></td>
</tr>
<tr>
<td>Malaise</td>
<td></td>
<td></td>
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<td>1/20 (5%)</td>
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<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>1/20 (5%)</td>
<td></td>
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<td>1/20 (5%)</td>
<td></td>
<td></td>
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<tr>
<td>Shock</td>
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<td>1/20 (5%)</td>
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<tr>
<td>Dizziness</td>
<td>1/20 (5%)</td>
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</tr>
<tr>
<td>Nausea</td>
<td>1/20 (5%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>Vomiting</td>
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<td>Abdominal pain</td>
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</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
<td></td>
<td>1/20 (5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hives</td>
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</tr>
<tr>
<td>Rash</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Reaction</td>
<td>5/20 (25%)</td>
<td>3/20 (15%)</td>
<td>3/20 (15%)</td>
<td>14/20 (70%)</td>
<td>7/20 (35%)</td>
<td>8/20 (40%)</td>
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</table>
Table 6  Reactions following ID primary immunization and a 2 year ID booster immunization.

<table>
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<th>Primary Immunization</th>
<th>2 yr. Booster I.D.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 Day 0 Day 7 Day 28</td>
<td>Day 7 Day 28 Day 28</td>
</tr>
<tr>
<td>60/81 (74%)</td>
<td>70/81 (86%)</td>
<td>57/81 (70%)</td>
</tr>
<tr>
<td>14/81 (17%)</td>
<td>17/81 (20%)</td>
<td>14/81 (17%)</td>
</tr>
<tr>
<td>25/81 (30%)</td>
<td>33/81 (40%)</td>
<td>27/81 (33%)</td>
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<tr>
<td>3/81 (3%)</td>
<td>4/81 (4%)</td>
<td>6/81 (7%)</td>
</tr>
<tr>
<td>22/81 (27%)</td>
<td>36/81 (44%)</td>
<td>31/81 (38%)</td>
</tr>
</tbody>
</table>

- Redness at inj. site
- Pain at inj. site
- Swelling at site
- Warmth at site
- Itching at site
- Itching generalized
- Fever
- Muscle aches
- Joint pain
- Lymph local
- Lymph syst.
- Malaise
- Headache
- Dizziness
- Hypotension
- Shock
- Vomiting
- Nausea
- Diarrhea
- Abdominal pain
- Hives
- Rash
- No reaction

| 57/81 (70%) | 1/81 (1%) | 1/81 (1%) |
| 1/81 (1%) | 5/77 (6%) | 1/77 (1%) |
| 47/77 (61%) | 24/77 (31%) | 5/77 (6%) |
| 69/77 (90%) | 30/77 (39%) | 2/77 (3%) |
| 77/81 (96%) | 57/81 (70%) | 1/81 (1%) |
| 57/81 (70%) | 1/81 (1%) | 1/81 (1%) |
| 1/81 (1%) | 1/77 (1%) | 1/81 (1%) |
| 31/81 (38%) | 1/81 (1%) | 1/81 (1%) |
| 24/81 (30%) | 11/81 (14%) | 12/81 (14%) |
TABLE 7  Reactions following ID primary immunization and a 2 year IM booster immunization.

GROUP B-2

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Primary Immunization</th>
<th>2 yr. Booster I.M.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
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<tr>
<td>Redness at inj. site</td>
<td>15/21 (71%)</td>
<td>18/21 (86%)</td>
</tr>
<tr>
<td>Pain at inj. site</td>
<td>2/21 (9%)</td>
<td>3/21 (14%)</td>
</tr>
<tr>
<td>Swelling at site</td>
<td>6/21 (29%)</td>
<td>8/21 (38%)</td>
</tr>
<tr>
<td>Warmth at site</td>
<td>1/21 (5%)</td>
<td>2/21 (9%)</td>
</tr>
<tr>
<td>Itching at site</td>
<td>6/21 (29%)</td>
<td>8/21 (38%)</td>
</tr>
<tr>
<td>Itching generalized</td>
<td>1/21 (5%)</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle aches</td>
<td>1/21 (5%)</td>
<td>1/21 (5%)</td>
</tr>
<tr>
<td>Joint pain</td>
<td></td>
<td></td>
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<tr>
<td>Lymph local</td>
<td>2/21 (9%)</td>
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<tr>
<td>Lymph Syst.</td>
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<tr>
<td>Malaise</td>
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<td>Hypotension</td>
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<td>Shock</td>
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<td>Headache</td>
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<td>Dizziness</td>
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<td>Fainting</td>
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<td>Nausea</td>
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<tr>
<td>Vomiting</td>
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<td>Abdominal pain</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Hives</td>
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<tr>
<td>Rash</td>
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</tr>
<tr>
<td>No reactions</td>
<td>6/21 (29%)</td>
<td>2/21 (9%)</td>
</tr>
</tbody>
</table>

A THREE YEAR STUDY
REPORT OF THE COMMITTEE ON RABIES

Chairman: Leon Russell, College Station, Texas
Vice Chairman: W. R. Miller, Beltsville, MD

W. H. Beckenhauer, NE; R. R. Brown, AR; Hans Draayer, IL; David W. Dreesen, GA; Thomas J. Galvin, DC; B. Hancock, IA; D. R. Howard, KS; Bruce Kaplan, KY; F. V. McCasland, TX; Robert B. Miller, MD; J. C. New, TN; J. C. Prucha, MD; E. L. Shroyer, OH; James M. Shuler, IN; Al Strating, NV; Taylor Woods, AR.

The Committee met on October 28, 1985, with 31 members and guests present.

Old Business

The current status on USDA, FSIS recommendations for management and slaughter of rabies exposed livestock was discussed. The current MPI Manual states that, “animals showing symptoms of rabies must be condemned. Animals bitten by a rabid animal must not be slaughtered for food purposes for at least eight months.”

In contrast to the official FSIS position, other government and public health agencies support alternative positions which most commonly require slaughter of exposed animals within seven days of being bitten. In addition, the latter alternate position usually includes excising liberal portions of tissue in the bite area on the animal at slaughter and encouraging the butchers to wear protective gloves.

Because progress is possibly being made towards change in USDA, FSIS policy on rabies exposed livestock, a motion was made and passed to table action on a corrective resolution until next year.

New Business

1. Dr. David Dreesen, Athens, Georgia, presented a paper, “Immune Complex-like Disease in Persons Following a Booster Dose of Human Diploid Cell Rabies Vaccine.”

2. Dr. Dennis Howard, Manhattan, Kansas, presented a paper, “The Kansas State University Three Year Study of Intradermal Human Diploid Cell Rabies Vaccine in Pre-exposure Prophylaxis.”

3. Dr. Dennis Howard also announced the initiation of a study on the use of VERO Cell Rabies Vaccine for pre-exposure prophylaxis.

4. Dr. Don Hildebrand, Athens, Georgia, presented a paper, “Studies on the Subcutaneous Route for Pre-exposure Rabies Prophylaxis in Horses.”

5. Dr. Richard Sharpee and Dr. Jay Gerber, Lincoln, Nebraska, presented a paper, “Stimulation of Cell Mediated Immune Response in Dogs, Cats, Cattle and Horses to Rabquard-TC Vaccine.”

6. Dr. R. B. Miller, Hyattsville, Maryland, presented a paper, “Current Regulations on Rabies Vaccines.”
7. Terminal discussion. Since many of the papers were interrelated, all the speakers were invited to answer questions on their papers or related areas. Special stated concerns were:

(a) lack of laboratories to do routine rabies antibody studies for people taking pre-exposure rabies prophylaxis;

(b) failure of all unvaccinated controls in vaccine studies to develop rabies when challenged;

(c) application of vaccine regulations to wildlife and exotic animal species;

(d) need for greater development of the cell mediated immune response stimulation index as a measure of immune response to rabies vaccines;

(e) need for a uniform policy for the management and slaughter of livestock exposed to rabies.

The Committee was adjourned.
SUMMARY
Serotyping of salmonella cultures from animal disease cases and epidemiologically related sources is reported for October 1, 1983, through September 30, 1984 (FY 1984). A total of 5391 cultures were serotyped. The most frequently identified serotypes were *Salmonella typhimurium*, *S. cholerasuis* var. kunzendorf, *S. heidelberg*, *S. typhimurium* var. copenhagen, and *Salmonella 18:Z4,Z32* (Arizona). The most frequent sources of cultures were cattle followed by turkeys, swine, and chickens.

INTRODUCTION
Data for this report were accumulated at the National Veterinary Services Laboratories, Animal and Plant Health Inspection Service (APHIS), USDA, Ames, Iowa. The data, except for serotyping results, were provided by the many laboratories requesting serotyping services. Most of these laboratories appreciate the importance of accurate data and made a concerted effort to provide quality input. Also, the reports were screened for obvious errors. However, it was not possible to verify each entry, and the quality of the total report is a reflection of the cooperative spirit of these laboratories.

The purpose of this report is to make the data available to epidemiologists and others who have a need for it. The data are presented in tables similar to previous reports in order that comparisons can be easily made. For this reason, although isolates formerly identified as “Arizona” are now reported on the basis of their corresponding salmonella antigens, they are separately reported in Tables 3 and 4.

DISCUSSION
Cultures were received for serotyping from 46 states, the District of Columbia, Puerto Rico, and Guam. This was one more state than in FY 83. The data for Guam does not appear in the tables.

A total of 5391 cultures were serotyped, which was less than the 5649 serotyped in FY 83, but close to the 5389 serotyped in FY 82.

*S. mbandaka* isolations nearly doubled from 38 in FY 1983 to 73 this year. The increase in this serotype was first noted in the 1981 report when there were 39 isolations with only 12 for the previous year. Isolates were received from 15 states. The majority of isolates were from miscellaneous
SALMONELLA SEROTYPES

sources (21), feed (16), cattle (13), and chickens (12) with fewer isolates from turkeys, ducks, horses, and the environment.

*S. sandiego* decreased from 164 isolations in FY 83 to 60 isolations in FY 84. The isolations continued to be made primarily from turkeys (83%) from California (65%).

*S. krefeld* increased from 67 isolations in FY 83 to 86 isolations in FY 84. As noted in the FY 83 report,² the majority of isolations continue to be made from horses (67%) from California (99%).

*S. muenchen* decreased to 45 isolations from 155 in FY 83 and 176 in FY 82. As noted in the FY 82 report,³ the majority of isolates that year were from cattle (59%) from Indiana (76%). In FY 83 cultures from cattle, swine, and horses from Indiana were about equal in number. Of the 45 cultures that were identified in FY 84, 20 were from cattle and the largest number of cultures from any one state came from Indiana (10/45).

Another serotype that increased this year was *S. java*. There were 43 total isolations from 7 states with 18 from swine, 8 from cattle, and 12 from other animals. There were only 6 isolates of this serotype last year, 9 in FY 82, 6 in FY 81, and 20 in FY 80.

Five serotypes were identified for the first time from animals in the United States. *S. kiambu* was isolated from a chicken-chill water sample. A parakeet from Illinois was the source of a culture identified as *S. ness-ziona*. *S. quiniela* was isolated from an owl in Louisiana. *S. roggeveld* was identified in a culture isolated from a turtle in California. A snake from Minnesota was the source of *S. windemere*.

REFERENCES


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<th>TABLE I</th>
<th>DISTRIBUTION OF SALMONELLA SEROTYPES BY STATE - FL64 (A)</th>
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<tbody>
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<td>ALABAMA</td>
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<td>ARKANSAS</td>
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<td>CALIFORNIA</td>
<td>COLORADO</td>
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<td>CONNECTICUT</td>
<td>DELAWARE</td>
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<td>DISTRICT OF COLUMBIA</td>
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<td>HAWAII</td>
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<td>INDIANA</td>
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<td>KANSAS</td>
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<td>WISCONSIN</td>
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<td>WYOMING</td>
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<td>State</td>
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</tbody>
</table>

**TABLE 1 CONTINUED**

| Serotype | Hartford | Havanna | Houten | Illinois | Indianapolis | Indiana | Inverness | Jamaica | Johannesberg | Heidelberg | Kentucky | Kralenberg | Krefeld | Lille | Livingston | Lexington | London | Madelie | Manhattan | Manila | Mbandaka | Meleacridis | Menhaden | Minnesota | Muenchen | Muenster | Neubrunswick | Newington | Neuport | Nienstedten | Pelham | Providence | Quakertown | Raleigh | Rolla | Salisbury | Santa | San Juans | Shakerh | Shreveport | St. Louis | St. Paul | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Louis | St. Lo
SALMONELLA SEROTYPES

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<td>TURKEY  CHICKEN  PIGEON  DUCK  CATTLE  SWINE  HORSE  SHEEP  SNAKE  REPTILE  ANIMALS  OTHER  OTHER  ENVIR.  MISCEL.  TOTAL</td>
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<td>TURKEY CHICKEN PIGEON DUCK CATTLE SWINE HORSE SHEEP SNAKE REPTILE ANIMALS OTHER OTHER ENVIR- MISCEL- FEED RONMENT LANY TOTAL</td>
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### Table 9. Horse--Most Frequently Identified Serotypes in FY84

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<th>Ave. Herd Size</th>
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*Number of times the serotype was identified

**Rank beginning with the most common

***Not previously included in this table
THE USE OF IRRADIATION FOR DESTROYING MICROORGANISMS IN MEAT AND POULTRY PRODUCTS

Ronald E. Engel, D.V.M., Ph.D.*

As part of its responsibility for ensuring that meat and poultry products are safe and wholesome, the Food and Safety and Inspection Service is beginning to undergo rulemaking for use of irradiation for fresh pork and preparing the organization for the eventual use in other red meat or poultry. In the U.S., food irradiation is regulated by the Food and Drug Administration, which by law must treat irradiation as a food additive; FSIS is required to act in agreement with FDA guidelines for its use. Some of the public health aspects of low dose irradiation, under 1 megarad, include:

- Irradiation of pork to control trichinosis
- Reduction of food-borne salmonella
- Reduction of parasites
- Reduction of nitrite used in processed meat products

Irradiation is not a panacea, but its potential for enhancing public health protection is bringing it to the forefront of discussion and regulatory action.

There is, of course, nothing new about radiation in itself: It is a purely natural phenomenon, always potentially available to man, that he has learned to harness. The scientific conception of radiation as a physical event within an atom — statistical, predictable, and measurable — is now nearly a century old.

Basically, radiation is the emission and propagation of waves or particles through space or material, the type of radiation most discussed for meat and poultry preservation is electromagnetic energy. The electromagnetic spectrum is divided into different groups based on wavelengths; the shorter wavelengths, for example, microwaves, ultraviolet rays, X-rays, and gamma rays, are the most damaging to microorganisms. Of primary interest for food preservation is electromagnetic radiations with wavelengths of 2,000 Å or less.

Simply put, gamma radiation consists of photons released from the nucleus of a radionuclide during its constant decay into a non-radioactive element. Radioisotopes release a fixed amount of energy. Cobalt 60 with a half-life of 5.2 years releases two gamma rays — one having 1.17 million electron volts [MeV] and the other 1.33 MeV per disintegration and Cesium 137 with a half-life of 30 years releases a photon of 0.66 MeV per disintegration. At these energy levels, it is not possible to activate any materials since the threshold for activation is about 10 MeV. Therefore, regardless of absorbed dose, the food product irradiated cannot become radioactive using Cobalt 60 or Cesium 137.

*Deputy Administrator for Science, Food Safety and Inspection Service, USDA
There are three basic terms employed to describe the various processes of food irradiation.

**RADAPPERTIZATION** — This process is equivalent to radiation sterilization or "commercial" sterility.

**RADICATION** — This process is equivalent to commercial pasteurization of milk. It reduces the number of viable specific nonspore-forming pathogens, other than viruses, so that none is detectable by available standards.

**RADURIZATION** — This process is also equivalent to pasteurization and involves enhancing the shelf life of a food by using the irradiation process to substantially reduce the number of specific spoilage organisms.

Applications of ionizing irradiation to foods are typically grouped in convenient dose ranges. A rad represents a fixed amount of energy absorbed per gram of matter. The absorber in this context is the food plus its packaging. Other terms in use include: kilorad — equal to 1000 rads; and megard — equal to 1 million rads. One kilogram is equal to 100 Krad.

The capability of ionizing radiation to accomplish chemical change is the key to the particular applications of irradiation to foods. The energy level of the radiation is usually great enough to break chemical bonds, resulting in physical and chemical changes, including the possible formation of radiolytic products. The nature of the radiolytic products which are produced depends on the chemical composition of the food. The concentration of these products generally increases with increasing radiation dose, but can be modified by factors during irradiation such as temperature, presence or absence of air, and the water content of the sample.

The radiolytic products also tend to be unevenly distributed in the treated food. More than 100 volatile compounds have been identified among the products of irradiation meat. However, none of these radiolytic products are unique. In experiments, approximately 90 percent of the radiolytic products that were identified were known natural components of food. Because the natural components of foods are now well characterized at the parts per million level, the remaining radiolytic products assumed to be unique may well be natural components of foods.

One important intrinsic advantage of irradiation is that the slight temperature increase during processing has little or no effect on heat-sensitive qualities of food such as flavor, texture, odor, and nutritional quality. This fact sharply distinguishes irradiation from other preservation techniques, such as thermal processing and freezing.

A possible area for the use of irradiation that has received considerable research attention is the low-dose treatment of fresh meats and poultry to extend freshness. Fresh meat and poultry products constitute the great bulk of meat consumed in the United States. The most common preservation techniques are the maintenance of cold storage and processing areas and delaying retail preparation to minimize the exposure of surface area to aerobic bacteria. Low-dose irradiation of fresh meat and poultry
products would delay microbial spoilage and extend handling life, thereby making possible a greater flexibility in distribution. There have also been studies of high dose procedures for processed meat products that would reduce the need for nitrite control for *C. botulinum*.

Of considerable interest is the use of irradiation to control bacteria that cause food poisoning. The most significant of these pathogens is *Salmonella*, which, as you know, is responsible for many of the cases of food poisoning reported in the United States. When considering *Salmonella*, *Campylobacter* and *Yersinia* must be included.

Theoretically, there are three possible approaches to the control of salmonellae in man: eradicating salmonellae in domestic livestock, training all food handlers in the proper techniques of sanitation (especially with poultry products), and the use of food irradiation. The first alternative is impractical at present. The second alternative would require a massive educational program to improve food-handling and sanitation practices. Total reliance on these measures against salmonellosis would be unrealistic. The third alternative, ionizing radiation, shows great promise for destroying a high percentage of *Salmonella* and spoilage microorganisms.

A considerable amount of research has been done on the irradiation of poultry carcasses to control salmonellae. One major study found that a dose of 250 Krad combined with a handling-environment temperature of 1.6° C resulted in a product that was essentially free of salmonellae and that could be kept safely, under refrigeration, for up to 20 days. This same study reported that higher doses would allow for a higher handling temperature but would result in color changes that could make the product undesirable.

In another study, a dose of 200-300 Krad has been found to be highly effective in destroying up to 3 log cycles of Salmonella, whether the birds had been chilled or deep frozen. Other nonsporing cells showed almost the same radiation sensitivity as salmonellae.

The primary microorganisms on refrigerated meats are species of the psychrophilic *Pseudomonas*. These gram-negative bacteria are among the more radiation-sensitive microorganisms. The reported doses required for 90 percent inactivation D-value of *Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* suspended in ground beef were found to be 12, 43, 58, and 64 Krad, respectively. Using low-dose irradiation on raw meat, the reported D-value for *Salmonella* species was from 55-78 Krad, for *Yersinia enterocolitica* 10-22 Krad, and for *Campylobacter jejuni* 14-16 Krad. Therefore, even a dose of 100 Krad or 1 KiloGray will result in up to 1.8 log reductions for *Salmonella*, 10 log reduction for *Yersinia* and 7 log reduction for *Campylobacter*.

On July 22, 1985, FDA approved the use of a dose from 30 to 100 Krad gamma irradiation for pork carcasses and fresh, non-heat-treated cuts for the purpose of trichinae control. In evaluating the pork petition, FDA considered data from a U.S. Army-USDA Agricultural Research Service sponsored study determining the wholesomeness of chicken parts ster-
ilized with a high Mrad dose of ionizing radiation. These studies — some of the most exhaustive wholesomeness studies ever undertaken on any food product — were designed to provide data on toxicity, carcinogenicity, mutagenicity, teratogenicity, and metabolite formation.

The toxicology studies of radappertized chicken strongly supported the safety of food irradiation. None of the diets used in the studies was mutagenic in the sex-linked recessive lethal test with fruit flies. No teratogenic response was found in any of the animal species. However, the mouse group receiving irradiated chicken was said to have had a statistically significant increase in the incidence of testicular tumors and lesions. In addition, there appeared to have been a reduction in the production of offspring in cultures of fruit flies reared on gamma-irradiated chicken diets.

With this information, FDA evaluated the relevant histopathology data from that study and did not find any treatment-related effect that is either biologically or statistically significant. The National Toxicology Program's Board of Scientific Counselors conducted — at FDA's request — a peer review of the relevant histopathology data, and concluded that the available data did not demonstrate a carcinogenic response.

By using low-dose irradiation some microorganisms will survive. Concern has been expressed that some of these survivors can undergo mutation from the initial exposure to the ionizing radiation treatment. There is no evidence at present that indicates such mutations can build up a resistance to the treatment or become virulent.

If the FDA were to clear the use of higher levels for chicken, pork, or beef, there would be added advantages to be given in respect to public health. A 250 Krad dose offers the following five advantages, some of which are not achieved by smaller doses:

- Reduction in fecal streptococci.
- Reduction of coliforms: Both fecal streptococci and coliforms are less than 1 per square centimeter of six carcasses
- Slight irradiation odor on the freshly treated raw carcass disappears after a few days storage, and gives way to a fresh chicken odor
- Continuing superior fresh appearance due to almost unnoticeable discoloration and low microbial counts
- Low cost to get maximum storage life.

Although there are many beneficial advantages to food preservation, there are several disadvantages and indeed some obstacles to food irradiation.

- The effect of flavor and texture is still undergoing research
- Acceptance of radiolytic products although in small quantities is still of concern.
- Cost and public acceptance are still obstacles.
There are some misconceptions that seem to be apparent to some uninformed groups.

- Food becomes radioactive — it does not
- Only irradiated causes bad chemical changes — it causes changes but no more than other processes such as cooking
- It forms sodium 24 — there is no induced radioactivity that can be detected above background level.

One of the primary interests of the Agency is to encourage the development of alternative methods of food preservation. We recognize that ionizing radiation holds great potential. Unquestionably, it offers many advantages to producers and consumers not offered by traditional methods.

The Agency is committed to moving as rapidly as possible to making this old technology an available technology for the meat and poultry industry. Issues such as dosimetry, quality assurance and labeling are undergoing review and will be resolved quickly and will be presented to the public for their comment in the very near future.
SUBTHERAPEUTIC USE OF ANTIBIOTICS IN FEEDS;
PUBLIC HEALTH CONCERNS

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It is a personal pleasure for me to address you today and to update you on the scientific and regulatory status of a most controversial issue, that being the use of subtherapeutic levels of antibiotics in animal feeds.

In a letter to Science in 1980, U.S. Representative John Dingell (D-MICH) stated with respect to the debate concerning the subtherapeutic use of antibiotics in animal feeds: “The science of this issue is well in hand, but we cannot call upon it to do the impossible. Twenty years of scientific investigation have identified but not quantified the risk to human health. We now face a fork in the road where prudent policy decision and not further study will be the pathfinder.”

There are several ways in which the feeding of antibiotics of animals may pose a potential health hazard to humans and other animals. First, pathogenic organisms such as Salmonella, existing in the GI tract of animals, can become resistant to the antibiotic(s) fed to the host animal at subtherapeutic levels and over time be passed into the environment and/or food to humans. Since the organisms are antibiotic resistant, if they produce clinical infection in humans or other animals, then the same antibiotic would be an ineffective treatment. Secondly, resistance that develops in non-pathogenic bacteria, for example, E. coli may be transferred to pathogenic bacteria either in animals or humans which may in turn cause a drug resistant infection. There is also concern that antibiotics in animal feeds may increase the prevalence or prolong the shedding of Salmonella organisms in animals, thus increasing the risk of disease in animals and humans.

FDA’s concerns regarding antibiotic resistance and the implications for human and animal health span some 30 years during which symposia, consultations with outside experts and task force reviews were held. Most notable among these actions was the establishment of the FDA Task Force on the Use of Antibiotics in Animal Feeds. Established in 1970 at the recommendation of FDA’s Science Advisory Committee, the Task Force was asked to undertake a comprehensive review of the use of antibiotics in animal feeds.

In its report issued in 1972, the Task Force acknowledged the potential human and animal health hazard of drug resistant bacteria and made a number of recommendations. In addition to basic research to better understand the nature of the problem, the Task Force recommended that restrictions be placed on the use of antibacterial agents in feeds which fail to meet guidelines established by the Task Force in regard to safety and/or efficacy. Agents that do not meet these standards would be prohibited from growth promotion and any subtherapeutic use in animals but could con-
continue to be used at therapeutic levels for short-term treatment on the order of licensed veterinarians.

Shortly after the Task Force made its recommendation, FDA in 1973 established a regulation that specified that antibiotics to be used in animal feeds for more than two weeks must meet the Task Force's criteria for safety in order to gain approval or to remain on the market.

A few years after the issuance of the Task Force Report, the Commissioner of the Food and Drug Administration (FDA) ordered an additional review of the data and the issues involved by the Agency's National Advisory Food and Drug Committee. This review involved public meetings and comments from all interested parties. After this review and taking into consideration the recommendations of the Advisory Committee, former Commissioner Donald Kennedy directed the Bureau of Veterinary Medicine [now the Center for Veterinary Medicine (CVM)] to publish a Notice of Opportunity for Hearing on a proposal to withdraw approval of New Animal Drug Applications for use of penicillin in animal feeds. This Notice published in the FEDERAL REGISTER on August 30, 1977, and was followed on October 21, 1977, by a similar Notice which proposed withdrawal of certain subtherapeutic uses of the tetracyclines, specifically Chlortetracycline and oxytetracycline in animal feeds. The manufacturers of the antibiotics requested a hearing.

Because of disagreement among some scientists as to whether the subtherapeutic use of these antibiotics results in significant health risks, Congress intervened and in 1979 directed FDA to contract with the National Academy of Sciences (NAS) to study the issues involved and earmarked $250,000 for that purpose. Congress also mandated that FDA hold in abeyance any implementation of its proposed actions pending final results of these studies.

In addition to the NAS report, other Congressionally mandated studies included the Office of Technology Assessment report on "Drugs in Livestock Feed" in June of 1979, and the USDA's report on the "Economic Effects of a Prohibition on the Use of Selected Animal Drugs" in December of 1978. These reports essentially supported the views held by FDA.

In March of 1980, the NAS submitted its report entitled "The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds." The report stated "the postulations concerning the hazards to human health that might result from the addition of subtherapeutic antimicrobials to feeds have been neither proven nor disproven. The lack of data linking human illness with subtherapeutic levels of antimicrobials must not be equated with proof that the proposed hazards do not exist. The research necessary to establish and measure a definite risk has not been conducted and, indeed, may not be possible." The NAS committee further concluded that it is not technically feasible to conduct a single comprehensive epidemiological study that will settle the issues. They offered suggestions for several less comprehensive, but more feasible, studies with the caveat that these studies had potential for clarifying certain points,
but would not settle the issues. They would, in essence, better define the links in the chain of events that is believed to exist from the feeding of subtherapeutic levels of antibiotics in animals to the development of drug resistant disease in humans.

In view of the NAS report, Congress, through the appropriations process for fiscal 1981, instructed FDA to conduct additional studies to generate new epidemiologic information consistent with the NAS suggestions and hold in abeyance any proposed actions until the studies are concluded. In response to the 1981 mandate of Congress to generate additional data, FDA awarded a contract to the Seattle-King County Department of Public Health to conduct an epidemiologic study of Salmonella and Campylobacter in commercial meat products in the community and their association with human disease. In August of 1984, CVM received the final study report and although it has been accepted as having met contractual obligations, the study is currently undergoing scientific review.

The predominant finding reported by the contractor in the food surveillance system was significant contamination of retail poultry by Campylobacter jejuni; 22.3% of specimens cultured C. jejuni while only 3.5% cultured Salmonella. The research contractor concluded that enteritis due to Campylobacter jejuni is more common than that due to Salmonella and that C. jejuni appears to flow from chickens to man via consumption of poultry products.

Considerable public attention has been focused on the antibiotics in animal feed issue as of late as a result of two recent reports from investigators at the Centers for Disease Control. One report in the August 24, 1984, issue of Science was a retrospective analysis of all CDC investigated Salmonella outbreaks during the 13 year period between 1971 and 1983. They discovered that in over two-thirds of U.S. outbreaks of multiple-drug-resistant Salmonella infections that had a defined source, such bacteria came from food animal populations. Animal origins were discovered more commonly in outbreaks involving antimicrobial-resistant Salmonella than in outbreaks involving antimicrobial-sensitive strains. In addition, the case fatality rate for patients with multiple resistant Salmonella infections was found to be 21 times higher than the case fatality rate associated with antimicrobial-sensitive Salmonella infections. Their assessment was that antimicrobial-resistant bacteria frequently arise from food animals and can cause serious infections in humans.

One of the major criticisms of FDA’s scientific basis for wanting to restrict the use of antibiotics in animal feeds have been that it has not provided any specific instances of human illness due to drug-resistant pathogens that resulted from the subtherapeutic feeding of antibiotics to animals. However, individual events in the complicated sequence have been documented. Another report by Dr. Scott Holmberg and others at CDC which appeared in the September 6, 1984, issue of the New England Journal of Medicine purportedly linked, for the first time, the use of
subtherapeutic antibiotics in livestock feed to the development of serious drug resistant infections in humans.

The article described the investigation of an outbreak of *Salmonella newport* involving 18 persons in the Midwest. The epidemic strain was resistant to ampicillin, carbenicillin, and tetracycline. Twelve of the patients had been taking penicillin derivatives for other medical problems. Eleven required hospitalization and there was one death. Through epidemiologic techniques and plasmid analysis, ground beef was implicated as the common food source of the infection and the meat was traced to cattle from a feedlot in South Dakota. The cattle had been fed subtherapeutic levels of Chlortetracycline for growth promotion and disease prevention. A major finding in this investigation was the identification of a segment of the population, i.e., those receiving antibiotics, that may be at higher risk of contracting a severe illness due to a resistant *Salmonella* infection. Presumably, the use of antimicrobials to which a pathogen is resistant would constitute selective pressure permitting the organism to flourish.

There have also been a number of other studies contracted for by FDA since the 1977 notices on the penicillins and the tetracyclines. These studies were designed to provide more information on specific segments in the postulated chain of events linking the subtherapeutic use of antibiotics in animals to the development of serious disease in humans. Notable among them was the work by Thomas O'Brien and colleagues the results of which were published in the New England Journal of Medicine in 1982\(^1\). This study provided for methodology developments that allowed one to determine whether or not plasmids from different sources (man and animal) were identical or similar.

The Center's belief that the continued unrestricted subtherapeutic use of these antibiotics presents risks to human and animal health is based upon consideration of a number of factors:

- Long-term, low-level feeding of penicillin and the tetracyclines promotes, by natural selection from the pool of normal intestinal flora, those enteric (gut) bacteria that contain R-plasmids. R-plasmids, also known as R-factors, are extrachromosomal genetic material which confer antibiotic resistance to host bacteria. These plasmids can be transferred between various kinds of bacteria through cell-to-cell contact (conjugation). Simultaneous resistance to several unrelated antibiotics is commonly carried on a single plasmid and therefore is simultaneously transferred from one bacterium to another.

- *E. Coli* strains bearing R-plasmids can be transferred from animal to man. Under the proper circumstances, organisms of animal origin can colonize in the human gut. However, colonization is not considered necessary for transfer of drug resistance to strains that inhabit the human gut.

- Use of penicillin and the tetracyclines also causes selection for pathogenicity factors, that is, disease-causing factors. These factors and
USE OF ANTIBIOTICS IN FEEDS

Drug resistance have been shown to be linked on the same plasmid. Pathogenicity and antibiotic resistance can therefore be transferred simultaneously to other organisms.

— R-plasmids can be transferred from normally nonpathogenic E. coli to certain pathogenic strains of bacteria with which they may come in contact in man or animals. Since R-plasmids carry drug resistance, this transfer can result in the creation of pathogenic strains of bacteria which are resistant to antibiotic therapy.

Continued unrestricted subtherapeutic use of antibiotics in animal feed increases the pool of drug-resistant bacteria in our environment. Moreover, the prospect of pathogens becoming drug resistant is, as FDA believes, a real threat to human health.

In a speech before the Congress in 1978, former Commissioner Donald Kennedy stated: "the evidence indicates that enteric microorganisms in animals and man, their R-plasmids, and human pathogens form a linked ecosystem of their own in which action at any one point can affect every other." If the vulnerability of microorganisms to antibiotics is reduced by the use of antibiotics for nonmedical purposes in animals, the effectiveness of medical treatment will be diminished in man. Potential risks to animal health also exist, and while the linkage to human health is indirect, animal agriculture faces the risk directly. The development of resistant strains, which is enhanced by subtherapeutic drug use, reduces the efficacy of those same drugs for the treatment of animal diseases.

On November 20, 1984, Secretary Heckler received from the Natural Resources Defense Council (NRDC) a petition to declare the subtherapeutic uses of penicillin and the tetracyclines in animal feeds an imminent hazard to the public health. NRDC argues that, on the basis of three recently published scientific studies — the O'Brien and the two Holmberg studies discussed earlier — FDA is likely to eventually withdraw approval of the subtherapeutic uses of penicillin and the tetracyclines in animal feeds. NRDC argues, based on these studies, that these uses meet the criteria for imminent hazard under the law. The petition and its impact were discussed before Congress, in hearing before the Committee on Science and Technology in December of 1984.

Before making any recommendation to FDA Commissioner Young and then to Secretary Heckler, the Center for Veterinary Medicine had to evaluate all available information, not just the three studies cited, before deciding on the petition. To assist in identifying pertinent available data and information, FDA decided to hold a legislative-type hearing on January 25, 1985, on the NIH Campus in which interested persons were invited to present their views.

Some 35 individuals representing industry, academia, government, consumers, agriculture, pharmaceutical manufactures, producers of red meat and poultry, and even a member of Congress spoke either for or against the NRDC imminent hazard proposal. Final comments were due in by February 11, 1985, and an official transcript was prepared.
The criteria used to evaluate the petition were the following:
- The likelihood that FDA will eventually withdraw approval;
- The severity of harm pending withdrawal of approval;
- The likelihood of harm pending withdrawal of approval;
- The risk to treated animals from suspended marketing; and
- Other approaches to protect the public health.

The NRDC petition was unique in that it involved an indirect effect, that is, the effect from the use of subtherapeutic levels of penicillin and the tetracyclines in animal feeds on the health of man. Previously submitted "imminent hazard" petitions dealt with direct effects as in the effect of a drug on a treated individual. Because of the indirect effect, demonstration of the harm to man is decidedly more difficult to measure. Quantitation indeed has been one of the major issues since the subtherapeutic use of antibiotics in feeds question arose in the 1950's.

NRDC estimated that between 100 and 300 deaths each year (depending on which of the provided estimates were used) may be attributable to the subtherapeutic use of penicillin and the tetracyclines in animal feeds. In addition, some 270,000 non-fatal cases of salmonellosis may also be due to the subtherapeutic use of antibiotics (penicillin and the tetracyclines) in animal feeds.

NRDC used Salmonella infections as the model to make their estimates of mortality and morbidity rates. They pointed out that these are conservative estimates (underestimates) because resistance also occurs in other pathogenic bacteria that cause human diseases. Some of the resistance in these other pathogens results from the pool of resistant bacteria in animals, which is ultimately due in large part to subtherapeutic use of penicillin and the tetracyclines in animal feeds.

NRDC concluded that there will be no significant negative effect on animal health from banning subtherapeutic uses of penicillin and the tetracyclines in animal feeds. They indicated that the use of these drugs for purposes of improving feed efficiency and weight gain is for economic reasons only and no health risks to animals will result if these uses are discontinued. The only potential animal health risk involves the use of these drugs for prevention of animal diseases. Since the petition is for suspending uses of penicillin and the tetracyclines, there are other antibiotics that can be used to prevent diseases. Also, there are effective alternatives to antibiotics, such as vaccines, to prevent diseases. NRDC also advocated changing certain farm management practices, such as reducing the crowding of animals in feedlots, which should reduce stress and transmission of diseases. Both of those actions it was said should reduce the need for disease prevention. NRDC pointed out that it is not advocating a ban of penicillin and the tetracyclines used at therapeutic levels to treat diseases.

NRDC also noted that the frequency of antibiotic resistance in bacteria that cause disease increases when animals are fed subtherapeutic levels of
these drugs. Thus, when animals become ill with one of these resistant organisms, treatment with therapeutic levels of the antibiotic of choice may not be effective.

NRDC contended that the suspension of these subtherapeutic uses of penicillin and the tetracyclines in animal feeds poses no human health problem. No potential human health problem has been identified in the literature. Any risk of eating meat from an animal that becomes ill, because penicillin and the tetracyclines were not available, could be alleviated by using substitute antibiotics and better farming practices to prevent or reduce the incidence of disease. Moreover, there would be an increased probability of effectively treating the disease with therapeutic levels of antibiotics if they were not used at subtherapeutic levels.

According to NRDC, the only possible impact of a ban on humans would be economic. A higher price for meat would be temporary. It was said that the average citizen consumes almost three times more meat per year than the U.S. Department of Agriculture considers necessary to meet nutritional needs. Thus, the consumption of a few pounds less meat per person per year because of economic reasons would not have any human health effect.

If the Agency decides to proceed with withdrawal, a formal evidentiary public hearing before an administrative law judge (ALJ) would be required. Under our law, such a hearing would be needed in this case even if the drug uses in question were to be found to be an imminent hazard. Granting an imminent hazard petition does not avoid formal proceedings. Rather, granting a petition suspends the marketing of a drug immediately — before the completion of the formal evidentiary public hearing, the ALJ's initial decision, and the Commissioner's final decision. Under the ordinary withdrawal procedures, in which a drug does not meet statutory requirements but does not present an imminent hazard, the drug may be marketed until the completion of all of these steps.

CONCLUSION

In an article written for the American Journal of Epidemiology, Reuel Stallones, Chairman of the NAS Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds stated: "Public policy and the actions stemming from it cannot always await the accumulation of scientific evidence and the development of prevailing views among scientists."

At the time of our original proposal to ban the subtherapeutic uses of penicillin and the tetracyclines in animal feeds, the contention was advanced that there were gaps in the scientific position to supporting the chain of events linking low level antibiotic feedings to disease in humans. Since then, newly generated data have been useful in filling these gaps in our knowledge. After FDA reviews and evaluates these new data, the Agency will know whether to proceed with the proposed ban.
In sum, two decisions must be made in the near future. First, whether the hazards to human health are of such significance as to call for an immediate ban of low level uses of penicillin and the tetracyclines in animal feeds, and whether to move forward with the withdrawal proceedings.

CVM is currently engaged in an active review of all available research and other information, particularly that generated since 1977, to assess the impact of this complex scientific issue on the subtherapeutic feeding of antibiotics to animals.

Thank you for allowing me to share these views with you and I am available for any questions that you may have.

REFERENCES


REPORT OF THE COMMITTEE ON SALMONELLA

Chairman: B. S. Pomeroy, St. Paul, MN
Vice Chairman: G. H. Snoeyenbos, Amherst, MA

C. W. Beard, GA; Fred D. Bisplinghoff, FL; B. O. Blackburn, IA; Tim E. Carpenter, CA; M. L. Crandall, MD; Larry E. Davis, IL; W. H. Dubbert, DC; Ray Fields, AL; R. D. Glock, AZ; R. W. Griffith, IA; D. A. Halvorson, MN; Wade L. Kadel, KY; D. D. King, MD; Glenn E. Klob, WI; T. T. Kramer, IA; C. L’Ecuyer, Canada; E. T. Mallinson, MD; C. S. McCain, OK; E. L. Menning, VA; W. Meyerholz, DC; C. D. Murphy, IA; K. V. Nagaraja, MN; Harvey J. Olander, CA; I. L. Peterson, MD; M. E. Potter, GA; R. A. Robinson, MN; Kathleen Sutch, IA; W. T. Tramel, MS; Keith Van Steenbergh, MO; S. A. Vezey, GA; M. W. Vorhies, SD; Ronald D. Welsh, TX; C. R. Weston, NH.
Ex-Officio: Alan H. Bentley, Canada; W. B. Bixler, MD.

The committee met at 1:30 p.m., Monday, October 26, 1985. Twenty-two members and 15 guests attended.

Six general reports were presented to the committee.

1. Dr. B.O. Blackburn reported for Kathleen Sutch and C.D. Murphy on Salmonella Serotypes from Animals and Related Sources Reported During Fiscal Year 1984 (Salmonella Typing Laboratory, NVSL). Serotyping of Salmonella cultures from animal disease cases and epidemiologically related sources reported for October, 1983 through September 30, 1984. A total of 5,391 cultures were serotyped. The most frequently identified serotypes were Salmonella typhimurium, S. choleraesuis var. kunzendorf, S. heidelberg, S. typhimurium var. copenhagen, and Salmonella 18:Z4,Z32 (Arizona). The most frequent sources of cultures were cattle, followed by turkeys, swine and chickens. Sutch and Murphy’s report will be published in the proceedings of this meeting.

2. Dr. C. L’Ecuyer, Health of Animals Directorate, Food Production and Inspection Branch, Agriculture Canada reported that the Canadian Salmonella control program has undergone a metamorphosis in the last year. In November of 1984 the Salmonella Co-ordinating Unit (SCU) under the direction of A.H. Bentley published its final document, A Report and Update on the Recommendations for the Control of Salmonella in Canadian Agri-food Industry. The Food Production and Inspection Branch of Agriculture Canada has accepted the succession of SCU and established a Salmonella and Food Borne Disease Control Program with a co-ordinating body in the Animal Health Division. They have no illusions as to the complexity of the task and the dedication and effort which will be required to make a significant impact on infections and contamination with Salmonella. However, they believe that the time has come to assist the producers in assuring the consumer of a safe supply of food products.
3. Dr. Fred D. Bisplinghoff (National Renderers Association) reported that Animal Protein Producers Industry Salmonella Committee decided its first activity would be to determine the current level of *Salmonella* recontamination in animal proteins. This will establish benchmark data which will indicate where the industry is now and the direction it should take in the future. All animal protein producing industry firms were asked to participate in this salmonella reduction program. Participating firms collected 75 gram samples of their finished product and submitted them to a qualified private laboratory for testing by AOAC method. Each firm collected five separate loading samples during a two week sampling period during July, August and September. Groups participating in the program are:

- A. Independent renderers
- B. Protein blenders
- C. Poultry processors
- D. Packer/Renders

Of 439 invitations to plants to participate, a total of 3,029 samples were received from 116 plants.

The APPI Salmonella Committee is in the process of establishing a meeting with USDA-ARS personnel to discuss joint funding of projects that would aid renderers in reducing salmonella recontamination of animal proteins.

Dr. W.H. Dubbert reported that USDA-FSIS is about to start sampling animal by-products from 174 packer/rendering facilities to determine current levels of contamination.

The Committee was very pleased with the progress the Animal Protein Producers Industry is making in the establishment of benchmark data. The project will be continued in January, February and March of 1986. The National Renderers Association is to be commended in taking the lead role in these efforts. USDA-FSIS is also commended in the development of a program to establish benchmark information on levels of salmonella contamination in packer/rendering facilities associated with animal and poultry processing plants.

Dr. Ray Fields of the Bureau of Commercial Fisheries, U.S. Department of Interior, indicated that the inspection of fishmeal plants and fishmeal was initiated in 1973. Inspection services consist of plant sanitation evaluations as well as sample collection and analysis of fishmeal for *Salmonella*. There are currently seven plants under inspection on the Gulf Coast and two on the Atlantic. Each plant is rated as Phase I, II, or III dependent upon *Salmonella* isolations. The incidence of *Salmonella* from all sample units in 1983 was 16.8%. Rates for 1984 and 1985 showed significant reductions to 6.1 and 7.0% respectively. At the end of the 1985 fishing year there are five plants in Phase II and four in Phase III.
Dr. R.A. Robinson reviewed the USDA Salmonella Surveillance Program. It is based on the submission of suspect *Salmonella* (and *Arizona*) cultures to NVSL at Ames, IA for confirmation and identification as to serotype. The program has been in existence for approximately 18 years. A minimum of epidemiological data is requested with each submission and based upon this data quarterly and annual reports showing the numbers of Salmonella serotypes by state of origin, source, species of animal or bird are compiled. Summary statistics also indicate most frequently isolated serotypes by species as well as herd size and mean morbidity and mortality data.

As a surveillance tool it is essentially a passive system with the potential for some biases. "Interest" factors can affect the overall data available, particularly as some submitting laboratories may feel qualified to identify common serotypes such as *Salmonella typhimurium* but not the less frequently isolated serotypes. While the turn around time at NVSL labs is rapid (4 days) many labs may submit cultures in batches, thus there is an unknown 'delay factor' in the system. Finally, submitting labs, because of time constraints or lack of interest by practitioners, may submit epidemiological data with the culture which is open to question as to its accuracy.

In order to make a surveillance system more responsive and hopefully more accurate, it may be useful to consider the following:

(a) Maybe have two classes of submissions
   (i) Cultures with an absolute minimum of data—e.g. species of origin, owner, veterinarian, name and address
   (ii) Cultures with more complete epidemiological information including existing data (herd size, morbidity and mortality, age group, primary/secondary infection) plus other relevant information on a species basis. For example, for swine we may wish to know the type of operation and housing, whether affected pigs were purchased or not, type of feed, etc.

(b) Using the existing microbiological techniques (such as serotype, phage typing, plasmid profile, etc.) to trace infections particularly those of considerable animal or human health implications, e.g. *S. dublin S. newport*.

(c) How to use the data base to bring to the attention of the various producer organizations the importance of Salmonellosis.

(d) How to make the service more attractive to current non-users.

(e) How to make more useful information available to practitioners and extension personnel. For example, the current data reports could be expanded to include more narrative reports on ongoing investigations and summaries of specific data analyses made by USDA or other researchers utilizing the data base.

(f) Continue efforts to monitor the accuracy of the data.
(g) And Finally, get USDA to recognize the importance of the problem by getting field epidemiologists interested in these projects.

5. Mr. S. Martin, CDC, Department of Health and Human Services, reported on the topic, "Serotype Specific Epidemiology of Salmonella. What Does it Mean?"

He reviewed the human *Salmonella* data for the past 17 years which revealed that there are specific epidemiologic characteristics that are unique for some serotypes. These distinctive distributions could be useful in designing evaluating prevention strategies if data became available on known or suspected vehicles for transmission of *Salmonella*. Therefore, data are needed on (1) consumption of beef, pork, poultry, dairy products, and other foods, by state, by age, and by season, and (2) occurrence of specific Salmonella serotypes among food animals.

Dr. M. Potter, CDC, commented on the Task Force Report on *Salmonella* Contamination of Milk in Illinois.

Late in March 1985, an unusually large number of culture-confirmed cases of salmonellosis in northern Illinois was reported. The dates of onset were ultimately shown to be between March 22 and mid-April, with two peaks in the occurrence of cases of March 29 and April 8. Initial epidemiologic investigations linked the outbreak to consumption of 2% lowfat milk produced by a large dairy in northern Illinois on March 20. As the investigations continued, it became clear that the second peak was associated with a different brand of 2% lowfat milk produced by the same dairy on March 30. When all of the epidemiologic and microbiologic data are considered, it appears probable that dairy products from multiple production days over many months were contaminated by the same clone of *Salmonella typhimurium*.

Because the milk coming into the dairy plant constituted a raw food of animal origin, it was initially considered likely that a persistently infected herd was the remote source of *S. typhimurium*; other possibilities considered included persistent or intermittent contamination of incoming milk by contaminated tankers, and a persistent focus within the plant. To test the hypothesis that raw products entering the plant were carrying the outbreak-related to *S. typhimurium*, records were reviewed to determine which sources supplied milk to the dairy plant on any of three days with known product contamination, a microbiologic survey was conducted of these sources. Between April 25 and June 14, milk from a total of 2,786 separate, indentifiable producers was tested, either as farm trucks unloaded or as over-the-road tankers loaded. Eleven (12%) of 94 larger composites from OTR's and 17 (4%) of 429 smaller composites from farm trucks were positive for *Salmonella*. Isolates represented 11 different serotypes plus an untypable group B, but the outbreak-related *S. typhimurium* was not identified in the raw product sources of the dairy plant. Although animal isolates of *S.
typhimurium for 1984–1985 were also obtained from NVSL to attempt to identify the remote source of the outbreak-related S. typhimurium, lack of bovine isolates from the two relevant states, Wisconsin and Illinois, frustrated this part of the investigations.

The rest of the investigations concentrated on determining factors that would permit Salmonella-contaminated raw products to get around or through the pasteurizers, and conditions that would permit the survival and/or amplification of Salmonella within the dairy. The task force reviewed a number of possible causes of contamination of 2% lowfat milk, but was unable to reconstruct an unbroken chain of probable events that would have led to intermittent contamination of pasteurized products with S. typhimurium. Based on the observed pattern of contamination of products and the data derived during the investigations, the task force feels that there was either an unidentified persistent focus within the plant environment, or intermittent reintroduction from a persistent focus outside the plant. The task force feels that the former is more likely. Means of contamination of 2% lowfat milk that could not be ruled out included cross-contamination of pasteurized product by co-mingling of raw and pasteurized milk in the skim milk transfer line, Salmonella present on threaded caps that are interchangeable between raw and pasteurized lines, and contamination of pasteurized product by reclaimed milk.

Dr. W. H. Dubbert, USDA-FSIS, presented to the committee a brief summary of the National Academy of Science's Report on FSIS activities. The NAS report acknowledged that, in general, the program had made progress in reducing risks to public health from conditions that can be observed during anti-mortem and post-mortem inspection and that a risk-based allocation of resources, supported by modern technology and a systematic evaluation of the programs would be valuable. It recommended that FSIS intensify its current efforts to control and eliminate microbial contamination, especially species of Salmonella and Campylobacter, explore rapid diagnostic procedures, continue to emphasize public education and seek better epidemiological procedures, continue to emphasize public education and seek better epidemiological surveillance.

The NRC report recommended that precepts of risk assessment (identification of the problem, exposure assessment, hazard assessment, and quantitative health risk assessment) be systematically embodied in the planning and evaluation of all phases of meat and poultry inspection and that risk assessment criteria be used regularly to assess consequences to public health of any modifications in the inspection process.

SUBCOMMITTEE REPORTS

A. Diagnostics, Data Collection and Epidemiology

The Committee recommended that the subcommittee review the
means of data collection and reporting and attempt to secure data from states doing serotyping that is not reported to NVSL Salmonella Typing Center.

It was further suggested that the subcommittee explore the possibility of issuing an annual report which includes isolations of salmonellae from man and other animals. This may be a joint effort of USDA and CDC.

B. Regulatory Programs

1. Production

Dr. I. L. Peterson, Chairman of the Subcommittee, reported the isolation of *S. pullorum* from 15 flocks from January 1 to October 1, 1985. Six of the isolates were from one state, the other nine isolates were from seven states and included an isolate from a parrot and two isolates from guinea fowl. All of the isolates were from small noncommercial flocks. *S. gallinarum* has not been reported since 1980.

Rhode Island and Kentucky were added to the previous 28 states classified as U.S. Pullorum-Typhoid Clean State. Two other states are expected to qualify for U.S. Pullorum-Typhoid Clean State-Turkeys, joining previously qualified California, Oregon and Washington. The Biennial National Plans Conference will be held in San Francisco, California June 24–26, 1986. Proposed changes in NPIP will be requested soon for consideration by the Conference.

2. Processing

Dr. W. H. Dubbert, Chairman of the Subcommittee, reported that FSIS is looking at incentive concepts to encourage industry use of the latest salmonella control technology. One way FSIS might positively reinforce plants that go beyond regulatory requirements in their salmonella control efforts is by permitting them to publicize this fact on their labels.

C. Industry

1. Feeds and Feed Ingredients

Dr. B. S. Pomeroy, Chairman of the Subcommittee, distributed a diagram of the procedure for the detection of salmonella in animal by-products that is being used in the APPI Salmonella survey. It was also pointed out that quality control laboratories for two turkey feed operations in Minnesota have found little improvement in the reduction of salmonella contamination of animal by-products over the past three years.

2. Poultry breeders

Carl Weston, Chairman, reported that efforts of chicken breeders to control salmonella are not encouraged by the lack of interest by the production industry. However, control efforts by primary breeders of turkeys are much more intensive and productive.
D. Research

1. Poultry

Dr. G. H. Snoeyenbos, Chairman, reported that little research was being conducted in poultry salmonellosis in the U.S.A. relative to the size of the industry and the magnitude of the infection. Work at the University of Minnesota continues on the evaluation of vaccines and testing procedures. A project on competitive exclusion has been initiated, in addition to that underway on feed pasteurization, at Russel Research Center, Athens, GA under the direction of Dr. K. Blankenship. Such research at the University of Massachusetts has been terminated following retirement of key personnel. Dr. S. Vezey, University of Georgia, is conducting investigations on foam vehicles for egg shell sanitizing.

E. Extension-Education

Dr. E. T. Mallinson, Chairman, reported that the subcommittee continues to seek ways to advance the development of effective education strategies and methods for improved biosecurity in food animal production. The subcommittee focus will be expanded beyond poultry production to include biosecurity and salmonella reduction aids targeted at livestock production, feeds and feed ingredients and the consumer. During the next year, subcommittee membership will be increased accordingly to include extension, private and industrial veterinarians with experience and interest in disease prevention and salmonella control in livestock. Representation on the subcommittee will also be sought from the feed and feed ingredients industry and FSIS.

The subcommittee believes that slide-tape sets and/or video cassettes similar to those developed by Agriculture Canada and other training materials need to be developed for use by food animal production groups in the United States. The initial attempt should be directed towards poultry production with educational aids for the production of other types of livestock following soon afterwards. Representation from APHIS, ARS, CDC, Cooperative Extension Service, FDA and FSIS was considered highly desirable along with the involvement of all or most subcommittee members and an educational psychologist. To ensure the timely, orderly reduction of these educational aids, the subcommittee recommends that their production development be coordinated by Dr. George W. Meyerholz, Program Leader, Veterinary Medicine, USDA Extension Service.

RESPONSE BY USDA TO RESOLUTIONS PASSED AT THE 1984 USAHA MEETING

1. Resolution concerned the establishment of a National Animal Salmonella Research Reference Center and an epidemiologist be added to the staff. USDA response was that National Animal Salmonella Reference Center at NVSL is provided and a trained epidemi-
ologist is available when salmonellosis outbreaks in poultry or livestock indicate the need for epidemiologic study.

2. Resolution concerned the five statements in respect to salmonella agreed to at the International Symposium on Salmonella, July 19–20, 1984, at New Orleans, Louisiana. The General Conference Committee of the NPIP endorsed the resolution and believes that this is a true consensus of salmonellosis in the United States and the world.

**COMMITTEE ACTION ON ONE PROPOSED RESOLUTION**

This resolution deals with the increase of the Poultry Diseases Budget, USDA-APHIS-VS to allow Federal Veterinary Medical Officers at the state level to be involved in field investigations of endemic avian diseases and to allow restaffing of regional poultry epidemiologists.

**SUBCOMMITTEE ASSIGNMENTS FOR 1985**

A. Diagnostics, Data Collection and Epidemiology
   Dr. C. D. Murphy, Chairman, Blackburn, Carpenter, Glock, Kadel, McCain, Potter, Robinson, Nagaraja, Vorhies, Welsh, Van Steenberg, Sutch.

B. Regulatory Programs
   1. Production—I. L. Peterson, Chairman, Beard, Crandall, Glock, King, Kolb, Mallinson, Menning, Nagaraja, Robinson, Snoeyenbos, Tramel, Vorhies, Weston, L'Ecuyer, Vezey.
   2. Processing—W. H. Dubbert, Chairman, Crandall, King, Kolb, Menning, Vezey.

C. Industry
   1. Feeds and Feed Ingredients
      B. S. Pomeroy, Chairman, Beard, Bisplinghoff, Crandall, Dubbert, Davis, Fields, King, Kolb, Nagaraja, Peterson, Robinson, Snoeyenbos, Vezey.
   2. Poultry Breeders
      C. R. Weston, Chairman, Halvorson, Nagaraja, Peterson, Kolb, Tramel, Snoeyenbos, Vezey.
   3. Livestock Industry

D. Research
   1. Poultry, G. H. Snoeyenbos, Chairman, Beard, King, Nagaraja, Weston.
   2. Swine, T. T. Kramer, Chairman, Glock, King, Olander, Robinson, Vorhies, Griffith.
   3. Cattle and Small Ruminants
      R. A. Robinson, Chairman, Glock, King, Van Steenberg, Vorhies, Welsh.
E. Extension-Education
   E. T. Mallinson, Chairman, Bentley, Carpenter, Crandall, Dubbert,
   Davis, Fields, Halvorson, Kolb, Meyerholz, Tramel, Vezey.
F. Ex-officio—Alan H. Bentley (Canada), W. B. Bixler.
Nematodirus battus: A Review of Recent Studies Relative to the Development of Surveillance and Control Programs

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The enteric nematode, Nematodirus battus Crofton and Thomas 1951, is the most pathogenic of helminth parasites infecting sheep (Ovis aries L.) in the Northern British Isles.\(^1\)-\(^4\) Historically this nematode and the associated disease, nematodiriasis [including disease caused by \(N.\) filicollis (Rulophi, 1810) and \(N.\) spathiger (Railliet, 1896)], was unknown prior to 1951. Since its discovery in the British Isles\(^5\),\(^6\) endemic populations of \(N.\) battus have been recognized in Norway,\(^7\) the Netherlands,\(^8\)\(^9\) Italy and possibly Yugoslavia.\(^10\),\(^11\) Limited foci of infection in Norway and the Netherlands became established following the importation of infected sheep from Great Britain.\(^7\)-\(^9\) However, the origin and abrupt appearance of \(N.\) battus in Great Britain (see Fig. 1) and other areas of Western Europe remains enigmatic.\(^12\) Here we report the first known occurrence of \(N.\) battus in the Western Hemisphere and present evidence suggesting this nematode was only recently introduced to North America. A review of available information, relevant in developing research programs for surveillance and control of this nematode, is presented.

HISTORY IN OREGON

Nematodirus battus is the only helminth parasite classified by the United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) and the United States Animal Health Association\(^13\),\(^14\) as an agent of a foreign animal disease in sheep. This important parasite was discovered for the first time in the Western Hemisphere in February 1985, from a flock of nine-month-old sheep, raised locally in the Willamette Valley of Western Oregon.\(^15\) These sheep were of mixed breed purchased from a local producer in Philomath, Oregon on 14 June 1984 to be utilized in an anthelmintic trial; maintained in pastures at the Veterinary Medical Isolation Laboratory at Oregon State University (OSU) until 5 October 1985; transferred to and confined in indoor isolation stalls until 12 and 19 November when they were necropsied. Small numbers of mature and gravid \(N.\) battus were recovered from four of ten sheep at necropsy. However, infections were dominated by two other species: \(N.\) filicollis and \(N.\) spathiger.\(^15\) Low numbers of reproductively mature parasites are usually associated with infections in older sheep where some degree of parasite resistance may have developed.\(^16\)-\(^18\)

The original diagnosis was confirmed by J. R. Lichtenfels (Biosystem-
atistics Parasitology Laboratory, USDA, Beltsville, Maryland) and officials at APHIS were immediately notified. Information, prepared by researchers at OSU concerning the presence of *N. battus*, was then disseminated to local producers and veterinarians by APHIS. This led to the identification of a second infected flock in May, 1985. A 12–16 week old lamb (born and raised on a farm bordering that from which the original Oregon isolate was obtained) developed signs of parasitic gastroenteritis, died, and was transported to our laboratory. Approximately 1500 *N. battus* were recovered from the small intestine of this animal. These accounted for 95% of the *Nematodirus* spp. present; however, only 6% of these were larvae. Postmortem examination revealed no gross evidence of other causes of death. In September, 1985, the presence of *N. battus* was confirmed (by fecal examination) in two, 7–8 month old lambs from a small flock at Toledo, Oregon, on the western slope of the coastal mountains about 50 miles from Philomath. This flock was also local in origin and had no history of introductions of animals from outside the area. Fecal samples from sheep at Ontario, Oregon (east of the Cascade Mountains) and from several other farms in the Willamette Valley were negative for *N. battus*.

In summary, the presence of *N. battus* has been confirmed in sheep from three flocks in Oregon (two from the Willamette Valley, one from the adjacent coastal zone). Infections have been found only in older lambs and no definitive cases of nematodiriasis have been identified.

**BACKGROUND**

In Great Britain, morbidity and mortality in lambs with nematodiriasis may approach 90% and 30%, respectively. Such devastating disease is typically seen during the spring in young lambs, 8–12 weeks in age. It is generally not seen in older animals. Disease is caused by penetration of the lamina propria of the small intestine by the larvae of *N. battus*. Tissue damage from this migration causes an acute enteritis, rapid weight loss, dehydration, and often death. The most severe outbreaks of disease reported to date have occurred in the British Isles and Norway.

*Nematodirus battus* may now pose as serious a threat to the sheep industry in North America as that which developed following the introduction of the liver fluke, *Fasciola hepatica* Linnaeus 1758, in the 17th century. Liver flukes continue to have a significant economic impact and are regarded as one of the most destructive helminth parasites of sheep in many areas of North America.

Control of *N. battus* in North America will depend on accurate methods for identifying the parasite and early knowledge of the local epizootiology. Male and female specimens have a characteristic morphology that readily

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*a* A preliminary bulletin concerning *N. battus* was prepared by us (EPH and GLZ) in March, 1985 and intended only for restricted use and distribution. Recently this manuscript was published without our knowledge in the October issue of the *National Wool Grower*. Significant editorial changes were made, and there were no attempts to contact the original authors nor Dr. D. Bailey who submitted our report.
distinguishes them from related species of *Nematodirus* (Figs. 2–5). Specific details pertaining to the differentiation of *N. battus* from other *Nematodirus* spp. endemic to North America have been presented elsewhere. Unfortunately, diagnosis based on the clinical evaluation of egg-morphology is often not possible and definitive diagnosis requires necropsy of sheep and recovery of identifiable helminths.

The life history of *N. battus* has unique aspects, when compared to that of other *Nematodirus* spp., which predispose this nematode to becoming widespread in North America. Infective larvae occur only during a brief period in the spring and usually just a single generation of parasite larvae emerge each year although variations have been reported. Larval development occurs entirely within the egg and hatching is inhibited until the larvae become preconditioned by exposure to cold temperatures and then warming to 10°C in a moist environment. These factors lead to a synchronous hatch, or pulse, of infective larvae in the early spring. Devastating disease outbreaks occur when young lambs ingest grasses heavily contaminated with infective larvae. Although transmission is typically from one year's lamb crop to lamb crops in subsequent years, older animals may harbor chronic infections. In Great Britain, the buildup of larvae to pathogenic proportions usually occurs in 2–3 year cycles. Although older animals may not be generally important in the yearly transmission of *N. battus*, they may be extremely important in transporting the parasite to new geographic areas.

The introduction of *N. battus* into Norway apparently resulted in the displacement of other endemic *Nematodirus* spp. in sheep (*N. filicollis* and *N. spathiger*) with it becoming the dominant species. This observation, along with the dominance of *N. battus* in Northern Britain, documents the important capacity of this parasite to displace other less pathogenic species. This is believed to be due to the specific *N. battus* life cycle which is tied to an array of environmental factors that can result in a burst of extremely high numbers of infective parasite larvae.

In retrospect we can document the presence of *N. battus* in North America dating back to 1983. Although its occurrence previous to then could have gone unrecognized, several factors support our contention that this is a recent introduction into the United States: 1) In Oregon sheep, *N. battus* co-occurred with *N. filicollis* and *N. spathiger* with the latter two species being dominant and this suggests recent co-existence of these three species. 2) Clearly defined clinical nematodiriasis has yet to be observed in Oregon. 3) Infections of *N. battus* have thus far been restricted to older sheep. 4) Sheep examined in our study were infected in the spring of 1984 and the population of *N. battus* larvae on the contaminated pastures had yet to attain concentrations high enough to be pathogenic. 5) No outbreaks of nematodiriasis were observed in the spring of 1985; however, one moribund lamb was found to have a gastroenteritis considered to be due to a heavy and nearly pure infection of *N. battus*. 6) Although we do not know at this time if other foci of infection exist in Oregon or in North America, it
NEMATODIRUS BATTUS: A REVIEW OF RECENT STUDIES

is unlikely that such a morphologically distinct and potentially pathogenic parasite could have remained unnoticed for any considerable time period.¹⁵

Environmental conditions in the Willamette Valley are similar to those of the British Isles and for this reason we believe that *N. battus* could easily become established as a major parasite of sheep in our region. With respect to this parasite, we have a dense and immunologically naive sheep population. This combination of host and environmental factors suggests that major disease outbreaks could occur especially in the less arid pasture lands of North America. The management practices of sheep producers throughout the Western United States include seasonal transportation of flocks from one area to another to take advantage of the most economical and available forage. As a consequence, failure to identify, contain and treat infected sheep and control use of contaminated pastures will insure rapid and widespread distribution of *N. battus* throughout North America.

RESEARCH REQUIREMENTS

DIAGNOSIS:

Accurate diagnosis is necessary to adequately assess the extent and level of infections in sheep. Negative fecal exams do not rule out the presence of *Nematodirus* spp. in either pre-patent or patent infections. Because immature worms do not lay eggs, fecal egg counts during the pre-patent period will be negative. Animals with patent *Nematodirus* infections may have negative fecal egg counts due to the low and sporadic numbers of eggs produced by the female worms. The McMaster technique cannot detect the low egg numbers that are often seen in *Nematodirus* infections; centrifugation/flotation techniques capable of detecting from 1 to 10 eggs per gram are preferrable. Diagnosis of acute cases during the pre-patent period currently relies on identification of worms originating in the small intestine. Once diarrhea has ensued, worms will often be passed posteriad into the large intestine or may even be expelled from the animal. Diagnostic characteristics of the eggs and adult worms are given in Figs. 2–5.

Recent advances in the immunodiagnosis of other parasites suggest that development of a rapid (conducted in the field) ELISA for detection of *N. battus* may be possible.

EPIZOOTIOLOGY:

Geographic Distribution: An active survey is needed to rapidly assess the extent of the distribution of *N. battus* in North America. Such a program should be comprehensive and adequately sample populations of sheep from a broad geographic area in the Pacific Northwest (Oregon,

ⁱ⁵The College of Veterinary Medicine, Oregon State University, the American Association of Veterinary Parasitologists, and Norden Laboratories have developed a chart, "Differential Diagnosis of *Nematodirus battus*,” as an aid to identification of this nematode.
Washington, Idaho, and northern California). Examination of rectal feces from sheep for eggs characteristic of *N. battus*, in conjunction with necropsy and identification of mature worms, would be appropriate. Sampling should include all age-classes of animals. The survey should initially concentrate on the Willamette Valley and adjacent areas of Oregon, expanding outward from identified foci. In other regions sampling could be accomplished at slaughterhouses or at auction, with a tracing of the history of any infected animals to determine their origin.

Knowledge of the geographic range of *N. battus* is important in understanding the extent of the potential problems that could arise from the presence of this nematode in North America. A broad distribution may indicate that the parasite has been present in North American sheep over an extended period. Conversely, a restricted distribution would suggest recent introduction and that populations of *N. battus* have yet to attain pathogenic levels in areas where the nematode is endemic.

**Patterns of Transmission:** It is necessary to establish if the patterns of infection, specifically factors influencing disease outbreaks and dissemination, are similar in northern Britain and Oregon. In particular, the following aspects must be determined:

1) Does the mode of transmission follow the typical pattern of spring infection or, additionally, can the cycle be completed in autumn? It has been shown in Britain and the Netherlands that seasonal variation does exist in the transmission of *N. battus*. This is largely due to eggs hatching in the same year as deposited, with infected larvae being present in autumn.

2) What is the survivability of eggs and larvae (over winter and through the summer) under environmental conditions in the Pacific Northwest? New data have recently been presented documenting the long-term survival of infective larvae of *N. battus* at varying temperatures.

3) Will populations of *N. battus* attain pathogenic levels under present management practices and climate in Oregon?

4) Are populations of *N. battus* maintained in reservoir hosts? Host records from Britain indicate this nematode is primarily a parasite of sheep. However, there have been reports from cattle, roe deer, and wild rabbits.

Knowledge of these aspects will allow an assessment of the potential impact of *N. battus* in sheep in North America. These studies could be conducted on pastures currently identified as being contaminated with *N. battus* (such as those at OSU). A sampling program utilizing parasitologically naive sheep as tracers in conjunction with herbage sampling for larvae is recommended.

**MONOSPECIFIC INFECTIONS:**

The establishment of sheep specifically infected with *N. battus* is necessary as a tool for other areas of research. Monospecifically infected sheep
will provide a source of adults and larvae (cultured from eggs) for use in 1) studies of comparative morphology and isoelectric focusing (IEF) and isoenzyme analysis, 2) experiments dealing with the pathogenicity of the parasites and for anthelmintic trials, and 3) development of a serological diagnostic test.

SYSTEMATICS:

Research in systematics involves the determination of relationships of organisms. Currently, techniques involving comparative morphology or biochemical analysis are involved in such studies.

Based on studies of comparative morphology, specimens from Oregon, Britain, and western Europe were indistinguishable. Consequently, work involving enzyme electrophoresis and IEF will be required.

Electrophoresis and IEF have proven to be reliable methods for the differentiation of species and strains of parasites. Comparison of enzyme profiles among populations of *N. battus* from North America and Britain and among other species of *Nematodirus* commonly occurring in sheep (*N. spathiger* and *N. filicollis*) are required to: 1) determine relationships of populations from these geographic regions, 2) determine relationships among focal populations in Oregon, 3) provide information to indicate how long the parasite has been present in North America (degree of divergence among populations), and 4) determine the evolutionary affinities of *N. battus* and other species common in North American sheep. This research will be enhanced by the development of monospecific isolates from Oregon sheep.

Further studies in comparative morphology will be needed to assess the overall evolutionary linkages among *Nematodirus* spp. in domestic and sylvatic ruminants throughout the world. These are particularly relevant as the phylogeny of *Nematodirus* spp. has never been determined and the relationships of *N. battus* remain completely unknown. Knowledge about the evolution of *N. battus* and related species will lead to an enhanced understanding of its epizootiology, particularly parasite-behavior relating to life cycles and pathogenicity in sheep.

ANTHELMINTICS:

Few anthelmintics have labeled approval for use in sheep. In practice, many are used on an "extra-label" basis. In general, nematodes of the genus *Nematodirus* tend to be the dose limiting parasites of most anthelmintics. Compounds such as Levamisole and thiabendazole are legally approved for use in sheep; however, their efficacies against *Nematodirus* spp. are lower than many recently developed compounds. Several of these new anthelmintics, although not available for sheep in the United States, have been shown to be effective against *N. battus* in other countries. Notably, both fenbendazole and oxfendazole have proven to be 100% effective (at 5 mg/kg) against immature and mature *N. battus*. The original discovery of *N. battus* in the United States occurred during an anthelmintic trial using Netobamine (Hapadex, Schering SCH 32481;
Nematodirus battus is present in the United States. Unique characteristics of the life cycle of parasites, particularly this species, require different approaches to surveillance and control programs than we are accustomed to applying to other disease agents. Parasitic helminth diseases do not behave as do viral, bacterial, protozoan, or arthropod diseases which can have explosive outbreaks after relatively short incubation periods. Relative to the susceptible host populations and the parasite population, the “incubation period” of a parasitic helminth disease can be years. In particular, for N. battus, a minimum of three years may be necessary for sufficient numbers of parasites to accumulate on contaminated pastures before there is a potential for clinical disease in the host population. Parasite populations may, additionally, be limited by extrinsic climatological factors. Thus, the occurrence of disease outbreaks is controlled by a complex series of events that are not often coincidental.

It is recommended that active, not passive, surveys be implemented to determine the extent of infection. Surveillance programs cannot be limited to young animals, nor should they be based only on fecal egg counts. Serologic tests may eventually prove to be the most accurate and economical means of diagnosis. Until effective anthelmintics are cleared for use in sheep, “extra-label” use of other highly efficacious compounds and management offer the best possibilities for control.

The occurrence of N. battus in North America could pose a serious threat to the sheep production industry. Consequently, it is important that the presence of this parasite in the United States not be met with complacency.
Fig. 1. History of geographic distribution of *N. battus* in the Holarctic. It is apparent that the distribution of this nematode has been influenced by the long-distance transport of infected adult sheep. a) First known occurrence in northern England in 1951, and later in Ireland.\(^3\)\(^5\) b) Introduced to Norway in 1961.\(^7\) c) First reported in the Netherlands in 1978.\(^8\)\(^9\) Development of populations of *N. battus* in these latter regions followed importation of infected sheep from Great Britain. d) *Nematodirus battus* has been known from limited foci in northern Italy since 1954 and possibly in Yugoslavia since 1963.\(^10\)\(^11\) However, the relationships of these apparently endemic populations of *N. battus* and others in western Europe remains unknown. e) Probable introduction into Western North America, ca. 1983. Populations of *N. battus* are currently known from three localities in Oregon.
Figures 2–5. Diagnostic characters for *Nematodirus battus*.

Fig. 2. Caudal extremity of female *N. battus* showing characteristic conical tail.

Fig. 3. Eggs of *N. battus*: a) *in utero*, b) from fecal exam. Note thick shell and polar-pitting (arrow) in latter.

Fig. 4. Copulatory bursa of male *N. battus* showing typical divergent lateral rays (arrow).

Fig. 5. Spicule-tips of *N. battus*, ventral view.
REFERENCES


RESEARCH OBJECTIVES TO IMPROVE U.S. DIAGNOSTIC CAPABILITIES FOR HEARTWATER DISEASE


ABSTRACT
The objectives of heartwater research at the Plum Island Animal Disease Center (PIADC) are to study the clinical aspects of Cowdria ruminantium in goats and mice, to develop methods to produce Cowdria antigen and to evaluate diagnostic techniques for Cowdria. Two mouse adapted strains of heartwater were received from South Africa in infected Amblyomma hebraeum ticks during 1983–84. A Caribbean strain from Guadeloupe was received in frozen goat blood in December 1983. Recently a Cowdria isolate was made at the PIADC from adult A. variegatum ticks received from the Central Veterinary Laboratory in Bamako, Mali. Techniques were developed to allow ticks to infect goats with heartwater. The clinical course of the disease produced by each of these four strains is being evaluated in both goats and mice. Preliminary trials indicate that the first three strains are antigenically different. An animal immune to one strain is not protected against a challenge with either of the other two strains. This antigenic variation could have significant importance when attempting to develop a diagnostic test or a vaccine.

A bank of sera from goats and mice recovered from first and second challenges and cross challenge studies has been established for future serological studies. Evaluation of the efficacy of fluorescent antibody or indirect fluorescent antibody tests for heartwater using mouse peritoneal macrophages as antigen is in the preliminary stages.

Several bovine and murine cell lines have been selected for attempted in vitro propagation of Cowdria. Pilot trials to grow Cowdria in vitro are in progress.

Colonies of A. hebraeum, A. variegatum and A. maculatum are being maintained for transmission studies and for developing means of detecting Cowdria infections in ticks.

INTRODUCTION
Recently three rhinoceroses from South Africa were imported into south Texas. When one of the animals died, a careful necropsy revealed toxic
hepatosis to have been the cause of death. An additional, and fortuitous, discovery was that the animal was infested with *Amblyomma hebraeum*, a vector of heartwater. Further examination revealed that the other two animals were also infested with *A. hebraeum* (Strickland, R. K., personal communication, 1984).

Heartwater, an often fatal rickettsial disease caused by *Cowdria ruminantium*, affects both wild and domestic ruminants. Many species of *Amblyomma* ticks may serve as vectors of heartwater, including two species found in the United States, *A. maculatum* and *A. cajennense* (Uilenberg, 1982, 1983). The worldwide distribution of *Amblyomma* ticks capable of transmitting *Cowdria* is far wider than that of the disease itself. In Sub-Saharan Africa, the mortality rate due to heartwater is unknown, but may be of equal or greater importance than trypanosomiasis to the livestock industry of many African countries.

*Amblyomma variegatum* ticks are believed to have been introduced with cattle imported into the Caribbean from West Africa in the 1800's (Uilenberg, 1983). Although Morel (1967) suspected that heartwater occurred in Guadeloupe in the 1960's, the conclusive diagnosis with the observation of *Cowdria* colonies in brain smears was not made until 1980 (Perreau et al., 1980). A joint United States-French-Dutch research project in the Caribbean has confirmed the presence of the disease on the Islands of Guadeloupe, Marie Galante and Antigua (Burridge et al., 1984). *Amblyomma variegatum* ticks have been found on 16 Caribbean Islands, and interviews with local veterinary personnel and farmers indicate that the distribution of *A. variegatum* has spread rapidly in recent years. The spread of *A. variegatum* has coincided with the increasing incidence of dermatophilosis an important skin disease of livestock (Burridge et al. 1984).

*Amblyomma* ticks may become infected with *Cowdria* as larva; nymphs or adults but do not pass the infection transovarially. *Amblyomma variegatum* ticks have a large host range that includes mammals, birds and reptiles (Hoogstraal, 1956). The possible importation of heartwater-infected *Amblyomma* ticks on migratory birds or infested exotic game animals poses a real threat to the United States livestock industry. The resulting problem would be two-fold: exotic ticks would be introduced onto the mainland, and these ticks could infect animals that in turn could serve as a means of infecting the domestic *Amblyomma* tick population.

Recent work at the Plum Island Animal Disease Center (PIADC) determined that white-tailed deer are susceptible to and die from heartwater (Dardiri, A. H., personal communication, 1984). Deer are commonly infested with *A. maculatum* in many southern states. A permanent cycle among wildlife, domestic ruminants and *Amblyomma* ticks could easily be established with the introduction of *Cowdria*-infected ticks.

In southern Africa, protection of susceptible livestock is accomplished by immunization with virulent *Cowdria* consisting of infected blood or tick stabilates followed by a treatment with tetracycline (Bezuidenhout, 1981). This system of vaccination is dangerous, costly, and would be too risky for
the United States as it might increase the chances of infecting our local tick population.

The threat of heartwater to the livestock population in the United States, the lack of a serological diagnostic test to detect infected animals, the unsuitability of available vaccines, and the failure of in vitro cultivation of Cowdria has made heartwater an important research priority in the U.S. Department of Agriculture.

**RESEARCH OBJECTIVES AT PIADC**

The objectives of heartwater research at PIADC are to study the clinical course of Cowdria ruminantium in goats and mice, to develop methods of producing Cowdria antigen, and to develop diagnostic techniques for Cowdria.

The Kwanyanga and Kumm strains of heartwater, both of which are mouse adapted, were requested and received from South Africa in infected A. hebraeum nymphs during 1983–84 (Table 1). The Gardel strain was received in August, 1984 in infected goat blood from the Institut d’Elevage et de Medicine Veterinaire des Pays Tropicaux (IEMVT), Guadeloupe in December 1983 (Table 1). In August, 1984, Cowdria was isolated from a tick stabulate made at PIADC from live adult A. variegatum received from the Central Veterinary Laboratory, Bamako, Mali. These ticks were collected on cattle near the Central Veterinary Laboratory at a site called Moribabougou (Table 1).

A wooden box (approximately $155 \times 76 \times 61$ cm) mounted on a metal frame was built to conduct tick transmission studies. This box serves as a self-contained stall and is equipped with a neck stanchion, food and water receptacles, and a drainage pipe. The box sits in a 15-cm deep metal pan filled with water that serves as a moat. To conduct transmission studies, a goat is placed in the box, its neck is locked into the stanchion and its hind legs are hobbled. An area is shaved on the lateral thorax just behind the scapula, washed, and a tick-feeding sleeve, constructed of chamois skin base and surgical stocknet sleeve, is glued in place. This sleeve is constructed by gluing a 30-cm section of surgical stocknet between two concentric rings of chamois skin. These rings are 18 cm in diameter with an inside circular opening of 8 cm. Once ticks are introduced into the sleeve, they can engorge on the goat, transmit heartwater, and be collected for further transmission studies. As a goat becomes clinically ill, its blood is collected in Sodium Heparin\(^1\) or Disodium Edetate Vacutainers\(^1\) and then injected intravenously (IV) into other goats or into mice IV or intraperitoneally (IP). Blood stabilates of the Cowdria isolates are produced from the goats by adding 10% Dimethyl Sulfoxide\(^2\) (DMSO) to collected blood and freezing immediately in liquid nitrogen.

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\(^1\)Becton-Dickinson, Rutherford, New Jersey

\(^2\)Fisher Scientific, Pittsburgh, Pennsylvania
Clinical disease has not occurred in PIADC Swiss mice injected either IV or IP with the Gardel or the Moribabougu strains. Both the Kwanyanga and Kumm strains are maintained in mice. Passages are done by killing a moribund mouse, homogenizing the liver in Snyder's media or phosphate-buffered saline (PBS) and injecting this homogenate either IV or IP into healthy mice. The Kwanyanga strain, injected IV, kills from 60 to 100% of mice. On some intraperitoneal passages of the Kwanyanga strains, a low percentage (10 to 30%) of mice become clinically ill. These mice may recover on their own or die. The Kumm strain, injected IV, usually kills 100% of mice. Clinical illness and death in mice injected IP with the Kumm strain varies greatly from passage to passage. As many as 100% of the mice may become ill, with many deaths (50 to 70%); on other passages only a few mice may become ill with no deaths. Both the Kwanyanga and Kumm strains can also be passaged using mouse blood. Mice that have recovered from the Kwanyanga or Kumm strain do not show clinical disease on a homologous challenge but will all die if exposed to a heterologous challenge with the other strain.

Peritoneal cells from mice infected IP with the Kumm strain can be collected by lavage. They are then deposited on microscope slides by cytospin centrifugation, fixed and stained with Giemsa. The morula stage of Cowdria can be seen in a low percentage of peritoneal macrophages. This type of antigen preparation has been used for an indirect fluorescent antibody test by du Plessis (1981). We are currently working with antigen preparations of this type in an effort to set up a serological screening test for heartwater detection.

Although the Kwanyanga strain occasionally causes mice to become ill by the intraperitoneal route of inoculation, no Cowdria colonies have been detected to date on Giemsa-stained preparations of peritoneal cells collected by lavage.

A study was done to determine which of 13 diluent would best preserve the viability of the Kwanyanga strain of Cowdria in mouse liver homogenate at room temperature. After maintenance of liver homogenate for 3 hours at room temperature, it was found that the use of Snyder's media resulted in significantly higher mortality in mice than did the more commonly used PBS or the buffered lactose peptone (BLP) (Birnie et al., 1984).

Both the Kwanyanga and Kumm strains are being maintained in A. hebraeum ticks. Amblyomma variegatum3 and A. maculatum4 have been infected with the Kwanyanga strains and are being used in transmission studies.

All four strains of heartwater held at PIADC (Table 1) have been used to infect goats and have caused high mortality. Necropsy findings have

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3Courtesy of G. Garris, ARS, Puerto Rico
4Courtesy of J. George, ARS, Kerrville, Texas
included pleural and pericardial effusion, pulmonary edema and positive brain smears. A study is in progress to compare the cross immunity of these four strains. The Kwanyanga and Kumm strains are antigenically different as are the Kumm and Garden strains. There appears to be cross immunity in goats recovered from the Gardel strain when challenged with the Kwanyanga strain. The Moribabougou isolate from Mali is being compared with the Kwanyanga, Kumm and Gardel strains to determine its relationship to them. This antigenic variation between strains may be highly significant when attempting to develop a diagnostic test or a vaccine. Consequently, a bank of sera from goats and mice has been collected during these studies for future serological testing.

On several occasions, buffy coat from an infected goat was separated on Ficoll-Paque grown in RPMI media supplemented with 10% fetal bovine serum and followed for 21 days. Cowdria was not successfully demonstrated in Giemsa-stained cell culture preparations.

Overlays of goat blood drawn from heartwater-infected goats during the febrile phase have not resulted in detectable infection in L cells or Vero cells. Mouse liver homogenate overlays on L cells or PU5 cells had similar results. Some initial co-cultivation studies using peritoneal cells collected by lavage with L cells with PU5 cells have not resulted in Cowdria infection. Cell cultures were monitored for cytopathic effect, by Giemsa stains of cell culture material and by mouse inoculation with cell culture material. Further efforts to culture Cowdria will be made using macrophage cell lines, bone marrow and various endothelial cells.

FUTURE NEEDS

A principal research goal for heartwater at PIADC is the adaptation of Cowdria to cell culture and/or the development of an alternative source of antigen. This initial, basic achievement will allow more sophisticated research to evolve; e.g., monoclonal antibody production, Avidin-Biotin detection of antigen in tissues, detection of antibody in sera, and purification of quantities of the organism and vaccine development.

Very little basic research on heartwater has been done outside of South Africa. The recent discovery of heartwater in the Caribbean has stimulated the interest not only of the U.S. Department of Agriculture but also of the Dutch, French and several universities in the United States. The fact that heartwater is a foreign animal disease precludes work being done in the United States at any site other than the Plum Island Animal Disease Center.

A great number of research problems remain to be resolved with heartwater. More personnel and resources are needed to resolve some of the

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5Pharmacia Fine Chemicals, Piscataway, NJ
6Courtesy of Walter Reed Army Institute of Research, Washington, D.C.
7Courtesy of M. Lawman, University of Florida, Gainesville, Florida
basic problems of this important disease. In addition, greater efforts are
needed to train veterinarians and livestock owners in the recognition and
diagnosis of heartwater. The United States Department of Interior cur-
rently oversees the importation of exotic game animals. Perhaps the
introduction of \textit{A. hebraeum} ticks will emphasize the importance of close
examination of imported game animals for ectoparasites of foreign animal
diseases.

The widespread distribution of \textit{A. variegatum} ticks in the Caribbean and
the known presence of heartwater on three Caribbean Islands as well as
tick-associated dermatophilosis represents a threat to the United States
mainland and also severely hampers livestock production in the Carib-
bean. An international campaign, with active United States’ involvement,
is urgently needed to eradicate \textit{A. variegatum} ticks from the Caribbean
Islands.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Barbara Moorhouse for her helpful
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REFERENCES

1. Bezuidenhout, J. D. 1981. The development of a new heartwater vac-
cine using \textit{Amblyomma hebraeum} nymphae infected with \textit{Cowdria
ruminantium}, In, \textit{Tick Biology and Control}, G. B. Whitehead and J. D.
Gibson, eds. Tick Research Unit, Rhodes University, Grahamstown,
Republic of South Africa, pp. 41–45.

diluents for maintaining the infectivity of \textit{Cowdria ruminantium}. Manu-
script in preparation.

3. Burridge, M. J., Barre, N., Birnie, E. F., Camus, E., and Uilenberg, G.
1984. Epidemiological studies on heartwater in the Caribbean with
observations on tick-associated bovine dermatophilosis. XIII World
Congress on Diseases of Cattle, Durban, Republic of South Africa.

4. du Plessis, J. L. 1981. The application of the indirect fluorescent
antibody test to the serology of heartwater. In, \textit{Tick Biology and
Control}, G. B. Whitehead and J. D. Gibson, eds., Tick Research Unit,
Rhodes University, Grahamstown, Republic of South Africa, pp. 47–52.

Department of the Navy, Bureau of Medicine and Surgery, U.S. Govt

Martinique. II. Agents pathogenic transmis par les tiques. Rev. Elev.

la cowdriose (heartwater) a \textit{Cowdria ruminantium} chez les ruminants


### TABLE 1

**ORIGIN OF THE FOUR STRAINS OF *COWDRIA RUMINANTIUM* USED AT THE AGRICULTURAL RESEARCH SERVICE, PLUM ISLAND ANIMAL DISEASE CENTER**

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>SOURCE/DATE RECEIVED</th>
<th>SUPPLIED BY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Kwanyanga</td>
<td><em>Amblyomma hebraeum</em></td>
<td>P. K. I. MacKenzie, Cooper (South Africa) (Pty) Ltd. Greensfields, Republic of South Africa</td>
</tr>
<tr>
<td></td>
<td>nymphs 830508</td>
<td></td>
</tr>
<tr>
<td>2. Kumm</td>
<td>mouse liver stabilate</td>
<td>J. L. du Plessis, Veterinary Research Institute, Ondersteppoort, Republic of South Africa</td>
</tr>
<tr>
<td></td>
<td><em>Amblyomma hebraeum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nymphs 831207, 840114</td>
<td></td>
</tr>
<tr>
<td>3. Gardel</td>
<td>blood stabilate</td>
<td>E. Camus, N. Barre, Institut d'Elevage et de Medicine Veterinaire des Pays Tropicaux, Guadeloupe</td>
</tr>
<tr>
<td></td>
<td>831221</td>
<td></td>
</tr>
<tr>
<td>4. Moribabougou</td>
<td><em>Amblyomma variegatum</em></td>
<td>M. Toure, T. Galvin, Laboratoire Central Veterinaire, Bamako, Mali</td>
</tr>
<tr>
<td></td>
<td>adults 840713</td>
<td></td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE ON SHEEP AND GOATS

Chairman: Michele C. Howard, Sacramento, CA
Vice Chairman: Jack R. Pitcher, Mission, TX

A. A. Anderson, IA; Miles H. Bairey, IA; W. W. Clark, MT; J. E. Fox, GA; R. F. Hau, GA; J. N. Huff, CO; Linda L. Logan, NY; Michael Marshall, UT; Blaine McGowan, CA; John H. Nieme, SD; James Quigley, GA; A. W. Smith, OR; Jeff Stott, CA; O. H. Timm, CA; Peter H. Timm, CA; R. M. Wainwright, NY; H. W. Whitford, TX.

The Sheep and Goat Committee met at 1:30 p.m. on Monday, October 28, 1985. There were 19 members and 42 guests in attendance for a total of 61 people.

The Committee met as requested by the President of USAHA to consider the business of the committee and submits the following report.

Dr. Gary L. Zimmerman, College of Veterinary Medicine, Oregon State University, presented a report on *Nematodirus battus* which in 1985 was identified as a parasite of sheep in the Willamette Valley of Western Oregon. This is the only nematode parasite of sheep that is listed by USDA and USAHA as an agent of a foreign animal disease.

This helminth is considered to be the most pathogenic parasite of lambs in Great Britain, where it was first described. Clinical disease may occur in up to 90% of the lambs in some flocks, with mortality approaching 30%.

The ability of *N. battus* to cause severe outbreaks of disease in lambs can be partially explained by the remarkable life cycle of the parasite. The egg, in which the larva develops to the infective third stage, has a remarkable ability to survive freezing, and can remain viable on pastures for up to two years. Cold weather is needed to sensitize the larva prior to hatching.

Dr. Zimmerman reported that no anthelmintic effective against this parasite is labeled for use in sheep at this time in the United States.

The Committee passed resolutions asking that USDA:
1. establish a surveillance program
2. develop improved diagnostic procedures
3. implement anthelmintic trials toward development of effective drugs to control this parasite.

Dr. Blaine McGowan, who has worked on footrot research at the University of California at Davis, for over 30 years, presented a report on *Producer Experiences with Footrot Vaccine*, authored by Dr. John Glenn, extension veterinarian at U.C. Davis.

Dr. McGowan stressed that the essential elements of footrot control still include proper foot trimming, footbathing, segregation of clean and infected animals, and culling of chronic carrier animals.

Field trials using the commercially available footrot vaccine have shown the vaccine reduces the occurrence of new cases in infected flocks and can accelerate the recovery of infected animals.
An informal survey of 25 producers who had used the vaccine revealed that vaccination of flocks on irrigated pastures seemingly resulted in a dramatic reduction in new cases of footrot as well as a dramatic improvement in infected animals. Owners of flocks running on dry range land also seem pleased with the vaccine. However, due to dry weather conditions, the challenge of these sheep was questionable.

The adverse effects reported by producers included reactions at the site of injection, especially when the vaccine was injected over lymph nodes or when the vaccine was given intramuscularly rather than subcutaneously. Reactions were very unpredictable in both occurrence and severity but appear to be more likely in black-face sheep and especially in black-face rams.

In a few cases producers felt there may have been systematic reactions such as transient fevers and even lameness. Several producers felt that the vaccination may have contributed to delayed conception in their flocks. However, none of these impressions have been confirmed.

Current recommendations concerning administration of the vaccine are:

1. to use special care in administering the vaccine to insure the injection is subcutaneous (18 gauge by 1/2" needle maximum) and not over lymph nodes, and
2. to avoid vaccination within 30 days of the start of the breeding season until the systemic reaction issue is investigated further.

Dr. McGowan emphasized the vaccine is not the sole solution to the footrot problem. The producer must continue with the basic footrot control procedures listed above.

In summary, there is a need for continued education on the importance of complete footrot control programs and a need for further assessment of any possible side effects of the vaccine.

The goal in every flock should be eradication rather than just reduction in the number of infected animals.

Dr. Michael Marshall, Utah State Veterinarian, reported that Utah State University is engaged in research on footrot vaccine.

Dr. Al Smith presented a brief overview of work on footrot vaccine, using progress at Oregon State University.

The Committee passed a resolution asking that USAHA assign to footrot a very high priority among diseases of sheep, and support funding of footrot research and vaccine development, at the highest possible level, for at least three to five years.

Also, that this resolution be referred to Agriculture Research Service and the Cooperative State Research Services.

Dr. Linda Logan, ARS, Greenport, NY, reported on improving U.S. diagnostic capabilities for Heartwater (Cowdria ruminantium). Dr. Logan reported that Amblyoma variegatum, one of the most common African
vectors, has been found on 16 Caribbean Islands, and that two U.S. species, A. maculatum and A. cajennense, have been shown to be suitable vectors.

The potential of accidental introduction of heartwater into the United States poses a serious threat to both our wildlife ruminants and domestic livestock.

Cowdria ruminantium is found in endothelial cells, and circulating and growing in neutrophils.

A research priority should be to provide a simple, reliable diagnostic test, to detect Cowdria antibody in animal sera, and Cowdria antibody in animal and tick tissues.

Dr. Jack Pitcher, Veterinary Services, Mission, TX, brought to the attention of the Committee, that in Fiscal Year 1984, Scrapie was reported in 23 sheep flocks in twelve states. Of these 23 outbreaks, eight flocks were totally depopulated, due to heavy infection. Total Federal indemnity paid during the fiscal year was $176,413.

During Fiscal Year 1985, 27 outbreaks were reported in 12 states. Of these, 14 flocks were totally depopulated, because of widespread infection. Federal indemnity paid during the fiscal year was $287,979.

The increase in reported outbreaks is believed to be due to improved reporting by flock owners because of the bloodline program.

The bloodline scrapie program came about as a result of a review conducted in 1982, because of the desire to reduce indemnity expenditures, and the concern expressed by industry, that some valuable bloodlines were being needlessly destroyed by scrapie eradication efforts.

It was the feeling of the Committee that a review of the scrapie eradication program was needed.

The embryo transfer project, a cooperative agreement between the International Sheep and Goat Institute/Utah State University and APHIS-Veterinary Services, is progressing. Embryo transfers in sheep have been completed. We are now at the stage of observing the resulting offspring for signs of scrapie, and are ready to move into embryo transfers in goats.

To date, no scrapie signs have been observed, in offspring produced from embryos transferred from scrapie inoculated ewes, to clean ewes, nor in offspring, resulting from embryos from clean ewes, transferred to scrapie inoculated ewes, and delivered by caesarian section.

The Scrapie Field Trial continues to supply tissues to research scientists working with slow viruses.

This research shows promise of developing information that may lead to a diagnostic tool to detect scrapie in the live animal.

The Committee passed a resolution requesting that APHIS conduct an in depth review of the bloodline scrapie program, to determine its effectiveness in obtaining eradication and the cost-benefit ratio of the program.
Dr. Darryl King, ARS, Beltsville, MD, discussed relocation of the Arthropod-Borne Animal Disease laboratory from Denver, CO to Laramie, WY.

Dr. King stated that a review team had investigated several sites and concluded that the University of Wyoming could provide suitable facilities at the lowest cost.

Thirty of the forty-five staff members are relocating to Laramie. Existing program funds will be utilized to cover the costs of moving. After this is accomplished, the research program will build back to nine to 12 scientists.

Containment research facilities and animal holding facilities will be rehabilitated by the University of Wyoming. ARS will reimburse the University on an annual basis. The program budget should remain at the same level.

The laboratory will continue to place emphasis on bluetongue and its vectors. However, this facility will not do any work on the development of vaccines.

Dr. King indicated that ARS would continue to collaborate with other agencies on existing vaccine research.

The goals and priorities of ARS's Bluetongue Research Program are to develop diagnostic tests to detect latent carriers of both Bluetongue and E. H. D. in order to get cattle back on the international export matter.

Dr. A. J. Luedke, ARS, discussed possible spread of Bluetongue Virus serotype 2 into Alabama. Dr. Lloyd Lauerman, Veterinary Diagnostic Laboratory, Auburn, Alabama, obtained BTV-2 from samples collected from cattle with clinical signs of bluetongue disease in West Central Alabama in July of 1984 and March 1985. Dr. Lauerman also isolated BTV-2 from a near-term aborted fetus in Southeast Alabama in January of 1985.

Genome changes appear to have occurred in the BTV-2 isolates, as the initial isolation from the Ona herd in September, 1982 was called Ona A. All subsequent isolations have been a different electropherotype which was called Ona B. This pherotype appears to be a highly variable BTV-2 genome of unpredictable pathogenicity.

The Committee resubmitted the two resolutions on BTV-2 passed in 1984, which asked USDA to continue the Florida sentinel cattle herd program, and extension of the sentinel cattle herd concept, into selected areas of the U.S. Last year APHIS did agree that a sentinel herd system, or some other means of surveillance, is desirable to monitor for the introduction of exotic bluetongue serotypes.

The Committee was apprised of the current status of last year's committee resolutions.

The Committee passed the seven resolutions outlined earlier, as well as a resolution asking that drugs approved for goats also be approved for sheep, making a total of eight resolutions.
APPLICATION OF MODERN BIOTECHNOLOGY TO THE DIAGNOSIS OF PARATUBERCULOSIS

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Paratuberculosis is a chronic debilitating infectious disease of ruminants that remains virtually undetectable clinically until the onset of a copious, nontreatable diarrhea and/or chronic weight loss and generalized unthriftiness. Paratuberculosis has been recognized in the United States and throughout the world since the early 1900's. However, there has been an increased awareness recently within the livestock industry as evidenced by the interest shown by major cattle magazines in their feature articles on paratuberculosis. As a result, the economic impact of the disease, the methods and implications of diagnosis, and the implementation of control and eradication programs are emerging as major concerns of international proportions.

The purpose of this report is to discuss the impact of modern biotechnological advances in research which are leading to improved identification of *Mycobacterium paratuberculosis* and offer promise for the development of sensitive and specific diagnostic methods. The close relationship of *M. paratuberculosis* to many other mycobacteria that are abundant in the environment emphasizes the need for definitive methods to identify clinical isolates and the requirement for *M. paratuberculosis*-specific antigens for the serodiagnosis of paratuberculosis.

**Identification of *M. paratuberculosis***.

Mycobacteria isolated from the tissues of animals with paratuberculosis are slow-growing, mycobactin-dependent organisms. It is generally accepted that mycobactin-dependence qualifies an organism to be identified as *M. paratuberculosis*,19 the putative etiological agent of paratuberculosis. However, mycobactin-dependence can no longer be accepted as the sole criterion for identifying an isolate because many strains of mycobactin-dependent *M. avium* have been isolated from domestic and wild animals.14,15,24 Biochemical testing of *M. paratuberculosis* is of limited value since there is variability in reactions between strains.8,19 Likewise, there is considerable variability in the gas-liquid chromatogram of mycobactin-dependent mycobacterial cell wall lipids;7 a species-specific fatty acid was not demonstrated in their studies. Serotyping schemes have been ineffective because the rough morphology of most strains causes autoagglutination. Antigenic analysis of *M. paratuberculosis* indicates that it is closely related to the *M. avium-intracellulare-scrofulaceum* (MAIS) complex.11,13,23

**Immunological diagnosis of paratuberculosis.**

The serological diagnosis of paratuberculosis has long been hampered
by a lack of specific and sensitive tests. Although a large battery of serological assays have been evaluated, circulating antibodies have generally been considered to be too low in titer, or too difficult to detect, or lack the necessary specificity to be of diagnostic value. False positive results are often the outcome, often due to using crude preparations of antigens that cross-react with other bacteria, or the use of antigens common to other species of mycobacteria.\textsuperscript{5,17,26} Allergic tests have also been evaluated, but suffer the same limitations of poor sensitivity and specificity. Immunological diagnostic methods have not been capable of differentiating infections due to \textit{M. paratuberculosis} from those caused by \textit{M. avium} or other unclassified mycobacteria.\textsuperscript{8,26}

\textbf{Serospecific glycolipid antigens of mycobacteria.}

Historically, efforts to identify immunologically active and specific diagnostic antigens have been focused on protein and polysaccharide antigens. To date, truly species specific protein antigens have not been identified within any member species of the \textit{Mycobacterium} genus although some individual proteins may contain epitopes specific for an individual species.\textsuperscript{12} On the other hand, work emanating from this laboratory has recognized the existence within individual mycobacteria of glycolipid antigens, relatively simple in structure, but with distinct species and subspecies specificity. Three such immunogenic glycolipid groups have been described.\textsuperscript{4} The first group, the polar C-mycoside glycopeptidolipid (GPL) antigens, are composed of a monoglycosylated fatty acylated peptide core which is further modified by small variable oligosaccharides. These small oligosaccharide groups (3–5 units) are responsible for the antigenic specificity of all serotypes in the MAIS serocomplex and some rapidly growing mycobacteria including \textit{M. chelonae} and \textit{M. peregrinum}.\textsuperscript{2,25} The second group, the lipooligosaccharides, are best represented in \textit{M. kansasii}. They present the unique immunochemical feature of a nonreducing trehalose substituent at the putative reducing end of the oligosaccharide moiety. The distal end, containing the novel epitope N-acylkanosaminyl(1→3) fucopyranose, is antigenic. The third group, the phenolic phthiocerol-containing glycosides, were identified in the course of a deliberate search in \textit{M. lepra} for a species-specific seroreactive glycolipid antigen. The phenolic glycolipid I from \textit{M. lepra}, containing the unique disaccharide determinant 3,6-di-O-methyl-β-D-glucopyranosyl (1→4)2,3-d-O-methyl-α-L-rhamnopyranose, is highly reactive and specific for sera from lepromatous leprosy patients.\textsuperscript{10} This disaccharide determinant has been chemically synthesized, coupled to bovine serum albumin as a carrier, and proven to be highly active in enzyme-linked immunosorbent assay (ELISA).\textsuperscript{9}

Schaefer\textsuperscript{21} observed that most nontuberculous mycobacteria have highly immunogenic species- or type-specific antigens and was able to devise a seroagglutination assay for the purposes of identification and classification. His procedure allowed recognition of at least 31 distinct serotypes within the MAIS complex. Serotyping these isolates has been important for epidemiologic and taxonomic purposes. Subsequently, it has
been proven that the Schaefer serotyping antigens were the first group of glycolipids mentioned above, the polar C-mycoside GPLs.\textsuperscript{2} Serotyping of strains has now been supplemented with chemical analysis of cell products. Thin layer chromatography (TLC) of lipid extracts from cells results in unique chromatographic profiles of polar GPLs, distinct for each serotype.\textsuperscript{5} The GPL serotype-specific antigens are also highly amenable to ELISA methodology.\textsuperscript{27} The ELISA can be used to assess the antigenicity of lipids, and as an adjunct to seroagglutination and TLC, for the identification of nontuberculous mycobacteria. As described above, a glycolipid antigen of \textit{M. leprae} has also been evaluated and proven to be a sensitive and accurate serodiagnostic antigen in ELISA.\textsuperscript{10}

In summary, the biotechnological advances in mycobacterial glycolipid antigen characterization have provided for identification of serotype- and species-specific antigens. These glycolipids have been purified and structurally characterized. As a result of detailed analysis, the antigenically specific determinants have been identified as residing in small oligosaccharide groups. The precise structure necessary for serological activity has been elucidated using chemically synthesized oligosaccharides and monoclonal antibodies. These discoveries made possible the chemical synthesis of an artificial antigen for the serodiagnosis of leprosy\textsuperscript{9} and identified specific monoclonal antibodies that can detect glycolipid antigen in serum of infected patients.\textsuperscript{3}

Development of an artificial antigen offers several advantages for the serodiagnosis of infection. Synthesis of only the serospecific determinant provides improved specificity because other possible cross-reacting determinants on the native macromolecular antigen are excluded. Production of an artificial antigen is particularly attractive for slow-growing or noncultivatable mycobacteria. Tedious purification of small amounts of antigen from infected tissues and cultures is avoided. Synthesis of the haptenic determinant allows attachment to various carrier substances, and thereby, the antigen is amenable to a variety of serological methods.

**Identification of GPL antigens from \textit{M. paratuberculosis}.*

Recently, we commenced a search for serospecific glycolipid antigens within \textit{M. paratuberculosis} isolates. A major immunoreactive glycopeptidolipid (GPL-I) was isolated and characterized by amino acid analysis, gas chromatography-mass spectroscopy, methylation analysis, and nuclear magnetic resonance spectroscopy.\textsuperscript{6} The glycolipid antigen belongs to the polar C-mycoside GPL family of antigens which are responsible for the serospecificity of all serotypes of the MAIS complex and are also found in \textit{M. chelonei}, \textit{M. peregrinum} and \textit{M. simiae} I and II. GPL-I could readily be detected by TLC; therefore, its presence offered hope as a phenotypic marker for identification of \textit{M. paratuberculosis} isolates. Recently, we showed that GPL-I was identical to the GPL which characterized serotype 2 of the MAIS complex (also called \textit{M. avium} 2) (R. T. Camphausen, R. L. Jones, and P. J. Brennan, submitted for publication). In addition, the TLC pattern of glycolipid antigens from other strains of \textit{M. paratuberculosis}
were analyzed and some were found to contain a major GPL antigen other than GPL-I, and again, a comparison of this GPL with the GPLs from members of the MAIS complex showed that it was identical to the GPL from serotype 8 of the MAIS complex (also called the Davis serotype or M. intracellulare serotype 8). (R. T. Camphausen, et al., submitted for publication). Accordingly, we now propose that some of the strains implicated in bovine paratuberculosis are closely related if not identical to some members of the ubiquitous M. avium/M. intracellulare serocomplex. However, the principle applies only to some isolates. Many other mycobactin-dependent fecal isolates do not yield a distinct GPL pattern and accordingly may not be related to the MAIS group.

In addition, antibodies specific for GPL-I (serotype 2) and serotype 8 GPL were detected in the serum of some paratuberculosis cows using an ELISA procedure. The presence of specific antibodies in the serum of cattle indicates that GPLs are immunoreactive antigens that cattle can recognize by producing specific antibodies. Therefore, the use of specific GPL antigens offers the promise of a serological tool for the specific diagnosis of bovine paratuberculosis. The suggestion that M. paratuberculosis exists in the form of serotypes with variable antigens provides the means for epidemiological characterization of paratuberculosis and calls for a re-evaluation of the taxonomic uniqueness of M. paratuberculosis.

**Etiology of paratuberculosis.**

The issue of classification of M. paratuberculosis and its differentiation from the MAIS complex has not been definitively addressed in the literature, but has been alluded to. Clearly, the identification of GPL antigens from M. paratuberculosis that are identical to the serospecific identifying GPLs of organisms in the MAIS complex renews this fundamental question. Antigenic similarities have been demonstrated between M. avium and M. paratuberculosis, including two M. paratuberculosis strains that were agglutinated by type-specific antiserum 2.11 Some strains have pathogenic characteristics of both M. avium and M. paratuberculosis.11 Mycobactin-dependence has been demonstrated within M. avium serotypes 1, 2, 3 and other untypeable strains.14 This mycobactin-dependence can be circumvented or lost in strains of M. avium and M. paratuberculosis.1,18,20 Further evidence of their close relatedness is the similarity of the extracellular iron-binding exochelins that they produce.1

Mycobactin-dependence does not appear to relate to pathogenicity for experimental animals.11 Similarly, paratuberculosis cannot be reproduced by many of the mycobactin-dependent organisms isolated from animals.14 Thus, there is the strong possibility that M. paratuberculosis, as presently recognized, represents strains of the MAIS complex that are organ-or host-adapted. Serotyping of MAIS complex strains has shown that bacteria classified in the same taxon may differ widely as host-adapted pathogens,11,28 and conversely, taxonomically distinct mycobacteria can produce similar lesions.16 Therefore, the singular criterion of mycobactin-dependence may identify many nonpathogenic as well as
pathogenic isolates as *M. paratuberculosis*. The close relationship of mycobactin-dependent strains to the MAIS complex, which are the dominant environmental agents of mycobacteriosis, suggests a need for further evaluation of the etiology and transmission of paratuberculosis.

**CONCLUSIONS**

1. Some strains of *M. paratuberculosis* produce sero-specific glycopeptidolipid antigens. In particular, two serologically distinct antigens have been identified in some *M. paratuberculosis* isolates and shown to correspond to the GPL antigens of *M. avium* 2 and *M. intracellulare* 8. This evidence may be the first ominous sign of a sero-complex of mycobactin-dependent mycobacteria.

2. The identification of serospecific GPL antigens offers promise of new tools to accurately identify and diagnose at least some *M. paratuberculosis* infections. GPL antigens can be detected by chemical or serological examination of cultures for species or subspecies specific identification. These antigens may provide the specificity needed in serodiagnostic tests since they are amenable to sensitive procedures such as the ELISA. Elucidation of the structure of the antigenically distinct oligosaccharides offers the possibility for synthesis of artificial antigens that could be readily available and highly specific. Production of monoclonal antibodies specific for the oligosaccharide determinants may provide an added approach to diagnosis of infection by allowing detection of the antigens in tissues or serum.

3. The close relationship of mycobactin-dependent mycobacteria to the MAIS complex, which are ubiquitous environmental organisms and opportunistic pathogens, suggests a cautious approach be taken towards the implementation of control and eradication programs until the etiology and pathogenesis of paratuberculosis is clearly delineated.

4. Modern biotechnological advances in research methodology have provided significant advances in our understanding of the antigenic relationship of *M. paratuberculosis* with other environmental mycobacteria. Glycolipid antigens have been identified which offer promise as tools to advance diagnostic, epidemiologic, and pathogenic studies of paratuberculosis. Further research should emphasize these areas.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


REPORT ON THE BOVINE TUBERCULOSIS OUTBREAK IN BISON IN THE UNITED STATES

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**C. D. Stumpff, D.V.M., Topeka, KS

SUMMARY

Only vestiges remain of an outbreak of bovine tuberculosis in bison in the United States that was first detected in April 1984. Movements of 485 tuberculosis exposed bison from two primary source herds in South Dakota traced to a total of 115 premises located in 24 states. A total of 21 infected herds located in 10 states were found as the result of the investigations of these movements. An additional 141 secondary exposed bison were traced to 35 owners from the 21 herds infected by primary source bison. No herds were found infected by these secondary movements.

Of 130 evaluations completed, 79 (61%) were made by the slaughter, with indemnity, and postmortem examination of tuberculosis exposed animals. Only 12 (9%) recipient herds containing 45 exposed bison received negative evaluations based on negative tuberculin tests of the exposed animals. The authors submit that in these few cases lie the most likely source of reoccurrence of bovine tuberculosis in the bison population in the future.

Epidemiology, pathogenesis, transmissibility, and tuberculin test efficacy for bovine tuberculosis in bison are discussed.

HISTORY

On April 4, 1984 a young bison bull with lesions of tuberculosis was found on regular kill in a small slaughtering establishment in South Dakota. The investigation of this case led to two bison herds in South Dakota in which bovine tuberculosis was confirmed, and to the first major outbreak of the disease in the United States involving bison. The tracing of tuberculosis exposed bison that moved from the primary source herds from January 1, 1981, led to the investigation of recipient herds located in 20 states. By September 30, 1984, the end of the fiscal year, 16 additional infected herds were found which were located in 9 states.1,2

PROGRESS IN 1985

The discovery of two herds which had received primary source bison in 1981, was cause enough to expand the tracing to include all animals that left these herds from January 1, 1978, the year in which the herds are believed to have become infected. This added about 100 bison and about 15 owners to the traceback list.

Five additional bison herds were found infected in fiscal year 1985 which brought to 21 the total number of infected herds found by tracing exposed

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**Regional Epidemiologist, USDA, APHIS, VS
bison from the two primary source herds. These five herds were located as follows: two in South Dakota and one each in Kansas, Minnesota, and Michigan. The addition of Michigan brought to 10 the number of states with infected bison herds. The total of 23 herds found to date are located as follows: five each in South Dakota and Colorado, three each in Kansas and Minnesota, two in Tennessee and one each in North Dakota, Nebraska, Michigan, Ohio, and Alabama (Figure 1).

A total of 626 tuberculosis exposed bison were traced to 136 owners in the United States plus one owner in Canada. Of these, most were primary traces from the original South Dakota herds; 485 bison to 115 owners. The balance were secondary traces from herds found infected by tracing primary source bison; 141 bison to 35 owners (Table 2). Figure 3 shows the distribution of tuberculosis exposed bison by states. Of the 35 recipients of secondary source bison, only 22 were new investigations, the balance of 13 owners having already been investigated for having received primary source bison. This became a problem in that some herds already evaluated for having received primary bison balked at undergoing a second evaluation (or third evaluation) as movement information developed from newly discovered infected herds. These double or triple “hit” [herds] account for most of the investigations that are still pending; i.e., two of six herds and 20 of 25 exposed bison not yet evaluated.

Evaluated herds fell into four categories, (1) infected, or (2) negative by single cervical tuberculin test of tuberculosis exposed animals, (3) negative by postmortem, histopathology and culture results of all exposed animals, and (4) negative by epidemiological considerations which was necessary when no exposed animals were available for evaluation.

By far the greatest number of evaluations were by the postmortem examination of exposed animals that were slaughtered without prior test, or by the slaughter of reactors to the single cervical test. Seven of the 21 infected herds were confirmed infected as the result of lesions found in TB REACTOR bison. The balance of 14 infected herds were found by the postmortem examination of non-tested exposed bison that were sent to slaughter with indemnity.

Of the 109 herds found negative, 60 were the result of negative postmortem and cultural findings in a total of 322 non-tested exposed bison slaughtered with indemnity. Negative epidemiological evaluation was the basis for clearing 37 herds. Twelve herds with a total of 45 tuberculosis exposed bison were cleared on the basis of negative single cervical tuberculin tests. Of these, seven herds contain 23 primary exposed bison, and 5 herds have a total of 22 secondary exposed bison (Table 4).

All infected bison herds were depopulated except for one municipal zoo herd of about six bison. In all, 2086 bison were slaughtered with indemnity of which 98 were reactors and 1988 were exposed. Cattle considered exposed to infected bison were also depopulated in most cases. A total of 878 cattle were depopulated as were the combined total of 42 swine and goats.
DISCUSSION:

The tuberculin test in bison showed high specificity in the limited testing done in this investigation. Eight (73%) of the total of 11 herds with tuberculin test positive exposed bison were confirmed as infected. A total of seven reactors in three herds were found without lesions on necropsy and were culture negative. Although this suggests that these reactors were not infected, it is more likely that these in fact were infected. Provided no other sources of exposure, the elimination of these non-lesioned reactors virtually assures bovine tuberculosis free status for the remaining bison of these three herds.

High sensitivity and high specificity of the single cervical tuberculin test were shown in one of the primary source herds where a total of 52 animals were cervical tested. Of these, 47 (90%) were test positive and 43 (91%) of these were found lesioned. Early reports of caudal fold tuberculin test results indicated that this test lacked sensitivity in bison, i.e., it did not detect infected animals with acceptable efficiency. Subsequent reports indicate that the sensitivity of this test is greater than was originally believed. However, too few caudal fold tuberculin tests were conducted in known infected herds in this outbreak to warrant an acceptable evaluation of the efficacy of this test in bison.

Susceptibility to the disease appears not to be as great in bison as was originally presumed. By far the most prevalent observed pathology involved the thoracic cavity and this almost always involved only the bronchial and/or mediastinal lymph nodes. Gross lung involvement or lesions of the parietal or pulmonary plura were rarely reported. Some cases of long standing were reported where the lesions had apparently healed. Only a few cases demonstrated abdominal lesions. In general, it appears that bison are more capable than cattle of resisting progressive disease following infection with \textit{M. bovis}.

Transmissibility of bovine tuberculosis was believed to be more efficient in bison than in cattle but this was not supported by field observations. In only one case of animals traced was significant spread shown to animals in the recipient herd. This herd of 18 original bison received 32 primary source bison in a single shipment of which 28 were found lesioned when slaughtered. All 18 original herd members were subsequently condemned for bovine tuberculosis at slaughter. Intraherd spread was also high in the two primary herds where postmortem findings of 466 animals evaluated revealed lesions indicative of tuberculosis in 232 (50%). Of the remaining 20 infected herds found, in none was it conclusively shown that bovine tuberculosis had spread from primary source bison to recipient herd members. Also, in no case was it conclusively shown that the disease had transmitted from bison to cattle.

Research currently in progress on bovine tuberculosis in bison is supported jointly by the National Buffalo Association, the American Buffalo Association, and by Veterinary Services, Animal and Plant Health Inspection Service. The research project began March 1985, at Iowa State Uni-
BOVINE TUBERCULOSIS OUTBREAK

versity. Experimentally infected bison and exposed cattle are confined under high biological security at National Animal Disease Center, Ames, Iowa. Negative control bison are located on a North Dakota ranch. The research will study pathogenesis of the disease in bison, transmissability of bovine tuberculosis from bison to cattle, the efficacy of the tuberculin skin test in bison, and procedures will be developed for the serological diagnosis of bovine tuberculosis in bison.

The results of investigations reported in this outbreak demonstrate clearly the value of the destruction of known tuberculosis exposed animals for the efficient detection of infection in recipient herds. Of the 14 herds found infected by the process of slaughter with indemnity of non-tested exposed animals, there is no doubt that some would have been missed if the determination were made by testing alone. Further, the 58 herds found negative by this process have the greatest assurance of true freedom from infection. Conversely, the 12 herds with 45 exposed bison cleared by negative tuberculin tests, provide the greatest potential for harboring *M. bovis* infected individuals. Such animals may be lesion free and test negative at this time but have the capability of developing progressive disease at any time for years to come. That so few exposed animals escaped slaughter provides some assurance against a repetition of this outbreak in the future.

This experience leaves no doubt concerning the exposure potential of the United States cattle industry to bison, and vice versa. In this respect, bison must be considered the same as cattle and not as an exotic species. Bison are held under conditions identical with beef cattle and often run as part of the cattle herd. In this outbreak, cattle exposed to tuberculous bison entered marketing channels and were traced to several cattle herds. In one case, five exposed Holstein heifers were traced to an embryo transplant station were they were purchased for use as surrogate dams. Other high risk situations were reported.

Finally, this outbreak provides a prototype whereby exotic diseases may find their way into the domestic cattle population. Bovine tuberculosis is believed to have been introduced to the two source herds by the purchase in 1978 of two sick elk from a small zoo. These elk died shortly after arrival and original resident elk began dying of an unknown cause which was shown later to be bovine tuberculosis. Unlike cattle, bison herds are often found on the same ranch with exotic animals. These exotic animals provide a direct link to exotic animal collections such as zoos, where diseases not commonly seen in domestic animals exist at higher prevalence. Bovine tuberculosis is an example of such a disease.

**BIBLIOGRAPHY**

Bovine Tuberculosis in Bison—FY 85

* States Receiving Tuberculosis Exposed Bison (24)

- Herds Initially Infected (2)
- Herds Found Infected In Fiscal Year 1984 (16)

☐ Tuberculosis Accredited-Free States Receiving Tuberculosis Exposed Bison (12)

▲ Herds Found Infected in Fiscal Year 1985 (5)
### Table 2

**Distribution of Tuberculosis Exposed Bison by Veterinary Services Regions**

**September 30, 1985**

<table>
<thead>
<tr>
<th>REGION (Number of States Involved)</th>
<th>TOTAL OWNERS REC'ING EXPOSED BISON</th>
<th>PRIMARY SOURCE EXPOSED BISON</th>
<th>SECONDARY SOURCE EXPOSED BISON</th>
<th>ORIGINAL HERD INVENTORY</th>
<th>STATUS OF HERDS</th>
<th>TOTAL DEPOPULATED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HERDS REC'ING</td>
<td>BISON PURCHASED</td>
<td>FOUND ON HAND</td>
<td>HERDS REC'ING</td>
<td>BISON REC'ED</td>
<td>FOUND ON HAND</td>
</tr>
<tr>
<td>NORTHERN (5)</td>
<td>11</td>
<td>11</td>
<td>62</td>
<td>42</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SOUTHEASTERN (2)</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CENTRAL (9)</td>
<td>93</td>
<td>82</td>
<td>347</td>
<td>259</td>
<td>21</td>
<td>59</td>
</tr>
<tr>
<td>WESTERN (8)</td>
<td>27</td>
<td>19</td>
<td>74</td>
<td>28</td>
<td>11</td>
<td>78</td>
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<tr>
<td><strong>TOTALS (24 STATES)</strong></td>
<td>136</td>
<td>115</td>
<td>485</td>
<td>331</td>
<td>35</td>
<td>141</td>
</tr>
</tbody>
</table>

| ORIGINAL SOURCE HERDS | 592 | 0 | 2 | 592 | 0 |
| GRAND TOTAL           | 3493| 5327 | 109 | 2219 | 928 |
DISTRIBUTION OF BOVINE TUBERCULOSIS
EXPOSED BISON
September 30, 1985

FIGURE 7

136 OWNERS
625 BISON

24 STATES
<table>
<thead>
<tr>
<th>REGION</th>
<th>TOTAL INVESTIGATIONS</th>
<th>HOW DETERMINED NEGATIVE</th>
<th>INVESTIGATIONS PPENDING</th>
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</thead>
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<tr>
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<td>POST MORTEM</td>
<td>NEGATIVE EPID.*</td>
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<tr>
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</tr>
<tr>
<td>TOTALS</td>
<td>136</td>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>

* EPIDEMIOLOGICAL DETERMINATION; NO EXPOSED BISON REMAINING ON FARM
DESIGN AND INTERPRETATION OF A SLAUGHTER SURVEY OF WISCONSIN CULL COWS TO ESTIMATE THE PREVALENCE OF MYcobacterium paratuberculosis INFECTION

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SUMMARY

Numerous slaughter surveys have been used to estimate prevalence rates of infection or frank disease, and attempt to follow its trends. Because the accuracy of the conclusions and predictions based on these surveys is influenced by many variables including sampling technique, specific source of animals, disease agent, factors affecting marketing such as economics and seasons of the year, the distribution of the disease, method of testing, and statistical design and interpretation, the influences of these variables on the results of this and other surveys were examined. Given the relatively low sensitivity of the culture method for diagnosis of subclinical infection with Mycobacterium paratuberculosis, statistical analyses were performed to aid in the construction and interpretation of the survey.

The ileocecal valve, ileocecal lymph node and cecal contents were collected from 205 Wisconsin cull cows at slaughter and cultured for M. paratuberculosis. Isolations of M. paratuberculosis were made from one or more of the 3 sites in 16 animals, resulting in an infection prevalence rate of 7.8% for this cohort. Shedding of M. paratuberculosis (positive culture of cecal contents) was observed in 4 of the 16 positive animals, i.e., 25% of infected animals and approximately 2% of the total animals sampled were shedding. None of the animals yielded M. paratuberculosis from only the cecal contents.

Since the actual prevalence of infection is only one of the many previously mentioned variables which influence the detected prevalence of infection, caution must be exercised in comparing results of different slaughter surveys and also in extrapolating the results of such surveys to larger more general populations of animals.

INTRODUCTION

Paratuberculosis, or Johne's disease, is an infectious chronic wasting disease of ruminants caused by M. paratuberculosis, characterized by a progressive diarrhea which is non-responsive to treatment. Diagnosis and control of this insidious disease are difficult due to numerous epidemiological factors, including: a long incubation period which can reach up to 15 years in length, a greater susceptibility of young animals as compared with adults, the rather nonspecific signs which result in a large number of differential diagnoses, the resistance of the bacteria to adverse environ-
mental conditions, as well as to common disinfectants, the absence of any effective treatment regimens, and the lack of a rapid and reliable diagnostic test.

The prevalence rates of *M. paratuberculosis* infection in various cohorts as determined by several survey methods have been used to estimate economic losses from the disease and justify control program rationales. The published narratives of many of the surveys in the United States do not include detailed descriptions of the survey design or analyses of the results. The primary objective of this study was to demonstrate how epidemiological and statistical concepts were used to design and interpret a slaughter survey for estimating the prevalence of *M. paratuberculosis* infection in a specific subset of Wisconsin cull cows. Numerous factors which influence the results of slaughter surveys were examined (Figure 1). In the survey, attempts were made to isolate *M. paratuberculosis* from ileocecal valves, ileocecal lymph nodes, and cecal contents. The distribution of isolations among the sampling sites and the relationships between these results were examined.

**MATERIALS AND METHODS**

**Survey Design**

The statistical and epidemiological methods used in the design of the survey are addressed in detail in DISCUSSION ON DESIGN.

**Animals and Sampling**

A slaughter plant which receives cull cows from a wide geographical area in Wisconsin was selected. An earlier slaughter survey for detection of *M. paratuberculosis* had been carried out at this same abattoir. Using a clutch-type sampling method, approximately 30 cull cows were sampled on each of 7 days over a period of 4 months, resulting in a total of 205 animals sampled. Sampling was performed on different days of the week and different times of the day. The sex and Wisconsin origin of the animals were verified by examining plant records. Other factors such as age and breed were determined by antemortem inspection of the animals. Access to individual animal identification was not permitted by the plant personnel.

The ileocecal valve, ileocecal lymph node and a portion of the cecal contents were collected from each animal, and placed in individual, labelled, sterile plastic bags so that the 3 samples from each animal could be identified. Instruments and gloves were cleaned between samplings to prevent any carryover. The samples were kept cold (but not frozen) during the period between collection and processing. All samples were processed within 6 hours of collection.

**Sample Processing and Culture**

Approximately 1 g of each sample of cecal contents was suspended in 25 ml of sterile water in an appropriately labelled screw-cap tube. The tubes were then shaken for 30 minutes and allowed to stand for another 30 minutes, after which 5 ml from the upper portion of the suspension was
transferred to a labeled screw-cap tube containing 25 ml of 0.25% hexadecylpyridinium chloride (HPC).

Tissue samples were processed in individual sterile plastic bags using a Stomacher blender (Stomacher Lab Blender 80, Dynatech Lab., Inc.). To process the ileocecal valves, a scraping was made of the mucosa using a separate utensil for each valve. Approximately 0.5 g of this mucosa was added to 10 ml HPC in the Stomacher bag. Each lymph node was incised with sterile scissors and 0.5 g of the parenchyma was removed and added to 10 ml HPC in a Stomacher bag. With both types of tissue, the contents in the bags were processed approximately 30 seconds in the blender, allowed to stand for another 30 seconds, the liquid portion decanted into the screw-cap tubes, the volume brought up to 25 ml with additional HPC, and the tubes shaken vigorously.

All samples in HPC tubes were held at room temperature for 48 hours. Approximately 0.3 ml of the resulting sediment was inoculated onto each of 2 slants of Herrold's egg medium with and one without Mycobactin-J.11 These were incubated in a horizontal position for one week in a 37°C high-humidity incubator, after which the tubes were examined for contamination, the caps tightened, and the tubes replaced in the incubator in a vertical position.

Cultures were examined every 4 weeks for contamination or *M. paratuberculosis* colonies. Bacteria from colonies with morphology compatible with *M. paratuberculosis* were stained using a modified Ziehl-Neelsen technique. When only one mycobactin-containing tube had acid-fast growth, or atypical colonial morphology was present, the growth was subcultured on slants of Herrold's egg medium with and without Mycobactin-J. Organisms which were acid-fast, mycobactin-dependent and required at least 8 weeks for visible growth were considered to be *M. paratuberculosis*.

**RESULTS**

*M. paratuberculosis* was identified in one or more sites from 16 of the 205 animals sampled (Table 1). Four animals were shedding the bacteria (here, defined as positive culture of cecal contents) compared to a total of 16 determined to be infected. None of the animals were found to be positive in only the cecal contents. Only 2 of the 16 culture positive cows were positive in the lymph node-only category. All of the isolations of *M. paratuberculosis* were made from samples taken on 2 of the 7 collection days. There was also a nonuniform distribution of the positive animals in relation to the markets which supplied the abattoir and no market was positive on more than a single day (Table 2).

**DISCUSSION ON DESIGN**

**Slaughter Plant Selection**

The national cow/calf population changes in a cyclical manner, peaking approximately every 10 years, with the general trend being to increase.
The number of cows slaughtered at federally inspected plants follows the cyclical pattern as well (Figure 2). The slaughter price tends to vary inversely with the number of animals slaughtered (Figure 3), as would be expected. These cyclical changes in populations and marketing patterns would be expected to influence the type of animals represented at slaughter plants. In addition, special programs, such as the recent dairy diversion program, influence the culling of animals from dairy herds and change marketing patterns.

Seasonal variations in marketing also occur which can influence survey results. For instance, data from the Market Cattle Testing program, which samples cows and bulls 2 years of age and older as a part of the State-Federal Cooperative Brucellosis Eradication Program, indicate that in Wisconsin over one-third of this cohort is marketed in the last quarter of the calendar year. Other seasonal influences, such as weather patterns, may change from year to year, influencing the availability of feed and thus affecting the culling patterns.

The geographical areas served by the various markets and abattoirs also influence the types of animals surveyed, and markets are not equally represented at the numerous slaughter plants in a state. Some markets characteristically sell cattle to one or two slaughter establishments, while others supply buyers from a number of plants.

The variable timetables of markets will be another one of the factors determining which markets are represented by a particular day's kill at a slaughter plant, and thus which populations of animals will be available for sampling. For example, if survey collections are routinely made on a Tuesday morning, the results may be drastically different from those obtained by sampling on a Friday afternoon, when a different population of animals may be represented. Although a slaughter plant may characteristically receive animals from a wide geographical distribution, if collections are always made on the same day of the week and time of the day, it may be found that in reality, only a single market was sampled. For that reason, the sample collections in this survey were made on different days of the week and at different times.

How then does one determine which slaughter plant(s) to use for the survey? What is required, is to be able to extract the maximum amount of information possible on the geographical source of the animals slaughtered. Since the results of any survey will be based on the actual disease prevalence and its geographical distribution; minimally, it needs to be determined whether the kill inventory tends to represent many herds or few, and from what general locale; and ideally, information on individual herd sources should be available. To obtain any of this information, the cooperation of the plant officials is essential. In this survey, information as to which markets were sampled was available by consulting the day's slaughter records in which the various lots (markets) and number of animals in each lot were identified. The plant, however, would not allow tracing back to the original herd of origin.
Animal Sampling

Once the plant is selected, how does one determine which cows are to be sampled? There are several methods or even combinations of methods to use. The samples could be collected randomly according to day and time, i.e., each animal would have an equal chance of being sampled. Clutch (or grab) sampling which is generally based on convenience, is characterized by obtaining samples from whatever animals are available at the time. By stratified sampling, i.e., attempting to obtain samples proportional to the various populations represented, an effort could be made to eliminate biases that may exist; but often this method may introduce other biases into the sampling system. In purposive selection, one could select for certain markets or types of animals in which one had particular interest. As previously described, in this survey a clutch-type sampling method was used, with sampling done on different days of the week and at various times of the day.

In many diseases, such as paratuberculosis, the characteristics of the animals sampled would significantly affect the results. Determinants such as age, sex, weight, vaccination status, stage of pregnancy, general health status, breed and other hereditary factors may influence the probability of disease but would not be readily apparent when only the viscera are available for observation. In this survey, antemortem inspection revealed the animals to be adult females, generally 1000 pounds or greater, of unknown paratuberculosis vaccination status, in various stages of pregnancy, of generally good health status (no condemned animals were sampled) and the majority were Holsteins.

Disease agent

Such characteristics of the disease as transmission, incubation period, pathogenesis, and virulence of the organism all influence whether infection occurred before or after the animals were assembled and thus affect survey design. For example, it has been suggested that swine exposed to erysipelas at an assembly point prior to slaughter may become infected resulting in an artificially high prevalence rate at slaughter. Similarly, it has been observed that the proportion of salmonella-infected swine is greater in the slaughterhouse holding pens than on the farm. Such observations would not be expected in surveys involving paratuberculosis because of its very different epidemiology.

Although the possibility of the passive excretion of M. paratuberculosis after ingestion in the market cannot be excluded, this seems not to be likely. Although the bacteria could be ingested under such circumstances, it is doubtful whether the concentration subsequently reached in the digestive tract would be detectable given the amount of feces produced by a bovine per day, and the detection capabilities of the culture method which begin at concentrations between 1 and 100 bacteria/ g of feces. In an earlier study, in which 300 fecal specimens from adult animals continuously exposed to M. paratuberculosis were cultured, the bacteria were isolated only 5 times. The results of this survey support the unlikelihood
of passive excretion, as there were no isolations of *M. paratuberculosis* from cecal contents only. Isolation of *M. paratuberculosis* at slaughter then, should be considered to be an indication of actual infection.

**Testing Methods**

The investigator must determine what will be used for the basis of positive and negative diagnoses having considered the sensitivity and specificity of the test method chosen. The primary purpose of this study was to determine the prevalence of bacteriologically demonstrable infection. At the time the survey was conducted, serological tests such as the AGID and ELISA were not recommended to be used for diagnoses in apparently healthy individual animals. The isolation of *M. paratuberculosis*, as defined previously in MATERIALS AND METHODS, was used as the diagnostic criterion, and is also the one currently being used by the Wisconsin Animal Health Laboratories. This diagnostic criterion was used with the understanding that other bacteria, such as some strains of the *M. avium-intracellularare* group, may have similar characteristics.

**Statistical Design**

After an initial determination has been made of the specific aims of the survey and the general methods to be used, a statistical design must be formulated. Given the specificity and sensitivity of the test considered for use, and an estimate of the prevalence of infection, what sample size would be necessary to result in statistically interpretable data and at what confidence level? Various combinations of sample sizes and prevalence will yield data with varying widths of confidence intervals (Table 3).

An example may help to illustrate this effect of sample size and prevalence on the confidence interval. Assuming the sensitivity of the culture method to be 40%, if the true prevalence of *M. paratuberculosis* infection was 10%, only a 4% (40% × 10%) infection rate would be detected. A 95% confidence interval can be thought of in the following manner: if 100 animals were sampled in each of 100 trials, one would expect that in 95 of those 100 trials, the detectable prevalence of infection would be in the range of 0–8%. If 200 animals were sampled in each of those 100 trials; in 95% of the cases, the detected prevalence rate would be expected to be in the range of 1–7%. This demonstrates that increasing the sample size results in decreasing the width of confidence intervals.

By extrapolation, if a detected prevalence of 4% was observed in a sampling of 100 animals, we could be 95% confident that the true prevalence rate was between 0–20%. This observation emphasizes that the detected prevalence must be interpreted as representing a range for the true prevalence rate.

The 95% confidence intervals for the 3 prevalence rates within the sample size category of n = 100 overlap, as do those for n = 200. Indeed, only for the sample size n = 1000 and prevalence rates of 10% and 20%, do we find that the 95% confidence intervals do not overlap. While this does not provide a formal test of significant difference, it does suggest that one
needs to use large sample sizes in order to detect even moderate differences in infection prevalence rates.

The next logical question is: what is the effect on the confidence intervals if the sensitivity of the culture method were twice as high, 80% for example? In that case, the width of the 95% confidence intervals would decrease. For example, for a true prevalence rate of 10%, a sample size of 200, and a test sensitivity of 40%, the confidence interval is 3–18% compared to 5–15% when the test sensitivity is 80%. Though the confidence intervals are narrower, it can be shown that with sample sizes of either n = 100 or n = 200, the 95% confidence intervals for prevalence rates of 5 and 10%, and 10 and 20% still overlap.

To assure the above calculations involving binomials are valid, key conditions must be met, including: all samples must result from independent selection, and the samples must be taken under the same circumstances. As the various slaughter surveys (including this one) rarely achieve these requirements, interpretation of results and comparison of results between different surveys is on shaky ground, at best.

The sample size is also influenced by laboratory capacity, among other considerations. In this survey, the capacity was limited to a total of 600 samples for culture. To obtain 600 samples, several regimens could be used. First, the collections of the 3 different specimens (valve, node, and cecal contents) could be made from 200 cows. Or, given the relatively low sensitivity of the culture method, duplicate cultures could be made from samples taken from 100 cows, or even triplicate cultures from 75 cows. Such replicate culturing, or statistical subsampling, can increase the number of isolations as demonstrated by Heard, et al. in isolating salmonellae from pig feces (Table 4): 8 positive specimens were cultured 12 times and only one specimen was positive on all 12 culture attempts.

To determine if replicate culturing would result in increased sensitivity for the Johne's survey described here, an analysis of the alternatives was conducted (Table 5). It is obvious that the difference in confidence intervals for the different regimens were minimal. For this reason, it was decided to culture 200 animals, thus permitting more markets to be represented. If capacity was not limited, a better option may have been to make triplicate cultures from 200 animals. Given a true prevalence of 10%, this would have resulted in a 95% confidence interval of 5–15%. (Note: all other parameters being equal, this is the same confidence interval as that resulting from single cultures of 200 animals and a test sensitivity of 80%.)

In summary, it has been shown that confidence intervals are relatively wide when disease prevalence can be decreased by sampling more animals, increasing the test sensitivity and test sensitivity are comparatively low. The width of the confidence intervals, and using statistical subsampling.

ANALYSIS AND INTERPRETATION OF RESULTS

The prevalence of *M. Paratuberculosis* infection in the group of cull cows studied in this survey was 7.8% (16 of 205). The prevalence of shedding of *M. paratuberculosis* was 2% of the animals sampled (4 of 205), and 25% (4
of 16) of the infected animals. This prevalence of shedding in infected animals corresponds to results of an earlier study (Buergelt, et al.) wherein 29% of proven infected animals had positive fecal cultures.³

Including lymph nodes in the sampling, vs. sampling ileocecal valves only resulted in only a 14% (2/14) increase in positive diagnoses, which is less than the 50% increase conjectured in an earlier study.² The total number of positive cultures, however, is too small to make any strong statistical statement concerning this observation.

Once the prevalence of infection is determined, there are various calculations that can be used to minimize bias in sampling or errors due to the testing procedure. For instance, if a test has a low specificity, especially when the real prevalence is low, a considerable error could be made in estimating the real prevalence. Methods of analysis are available for minimizing such error.⁹

In retrospect, it was advantageous that the regimen of sampling 200 animals was selected, for this allowed a larger number of markets to be represented and a clustering of positive culture results by markets and days was observed. Excluding private lots, 4 of 9 markets were responsible for supplying all the animals from which M. paratuberculosis was isolated (Table 2). Although Market A supplied over twice as many animals as any other market and was represented on 5 different collection days, there was only 1 isolate from its animals. Market B, the next largest supplier, had the largest number of positive animals and all of those occurred on one day's collections. Other markets were represented on only one sampling day and characteristically had either high or low prevalence rates. Because of the small number of sampling days, speculations as to the probability of finding consistently high or low prevalence rates for any specific market can not be made. If upon repeated sampling, however, some markets were shown to have consistently high or low rates, the effect of those rates on the overall prevalence rate could be evaluated, taking into account the degree of representation of that market at the slaughter plant.

One logical explanation for the observed clustering would be the fact that all the positive animals from a market on a certain day were from the same herd. Also, it should be considered that the infection in Wisconsin is, quite probably, not uniformly distributed geographically. Hence, markets located in areas with a high number of infected herds may tend to receive a greater number of infected animals.

Contributions to apparent clustering could be from such points of technique as: bacterial carryover on equipment as samples are collected, or carryover in the laboratory as samples are processed. This would increase the number of positive results. Such errors were minimized in this particular survey by sanitization of the equipment between collections, utilization of separate or sterilized equipment in the processing of samples, and employment of the same personnel for collection and processing of samples. Errors in processing or media formulation could result in false negatives. Such errors were unlikely in this survey, as clustering was not
observed in the private diagnostic samples submitted to the same laboratory as the survey samples, and processed in a similar manner.

As has been shown, there are numerous factors contributing to prevalence of infection determined by a slaughter survey. The difference between the prevalence rate of 10.8% from an earlier Wisconsin survey and 7.8% from this one is probably more apparent than real, as it has been shown that the confidence intervals are very wide. To attempt to attribute the difference of 3% to any factor(s), including actual prevalence of infection, would be a meaningless exercise. It would also be dangerous to try to base any conclusion about disease trends on such survey results.

An infection prevalence rate can only be applied to the cohort from which the samples for the determination were taken. Therefore, attempts to correlate the Wisconsin surveys with others such as the New England survey (Chiodini, et al.) or an earlier survey in Denmark would be erroneous since the cohorts, as well as the survey methods, were different. Because culled cows are not representative of the cattle population as a whole, extrapolating the prevalence rate of a disease as estimated by a limited slaughter survey to that of the general population would, again, not be valid.

Because of the multiplicity and interrelationships of the variables influencing the universe of animals from which slaughter survey samples are drawn, it is almost impossible to design an "ideal" survey which takes all the influences into account. Notwithstanding the limitations inherent in slaughter surveys, they do have utility. They can be used to demonstrate that infection in fact, exists. If done on a sufficiently large scale, they can identify trends and can be used as a surveillance tool, as in the State-Federal Cooperative Brucellosis Eradication Program. In national disease surveillance programs such as those in New Zealand and Denmark, they may aid in demonstrating temporal and regional differences as well as aid in the determination of economic losses. Slaughter surveys can also provide a means to determine which tissues, procedures, etc., are the most efficient to use in the diagnosis of a disease, as in the case of isolation of *Yersinia spp.* from swine. Therefore, slaughter surveys which are adequately designed and interpreted will continue to be a valuable tool for the epidemiologist, regulatory and private veterinarians, and the livestock industry as a whole.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Brian S. Yandell, Departments of Horticulture and of Statistics, University of Wisconsin-Madison, for his advice on statistical design and analysis, which was essential to this survey; the slaughter plant personnel, who wish to remain anonymous; and the Wisconsin Central and Regional Animal Health Laboratories for their technical guidance and media preparation.
BIBLIOGRAPHY


17. Personal observation.


Figure 1. Some Factors Affecting Slaughter Survey Results

- Geographic Distribution
  - Source Animal
    - Age
    - Sex
    - Herd Status
    - Weight
    - Breed
    - Pregnancy Status
    - Health Status
    - Vaccination Status

- Sampling Technique
  - Random
  - Grab
  - Stratified
  - Purposive

- Marketing Influences
  - Economics
  - Population Dynamics
  - Date, Time, etc.
  - Market/Plant Location

- Statistical Design and Analysis
  - Sample Size
  - Confidence Intervals
  - Replicate Testing

- True Prevalence
  - Disease Agent
    - Virulence
    - Concentration
    - Tissue Location
    - Transmission

- Diagnostic Procedure
  - Sensitivity
  - Specificity
Figure 1. Cows Slaughtered vs. Cow/Calf Population

Source: Livestock and Meat Statistics
U.S. Dept. of Agriculture

Figure 2. Number Cows Slaughtered vs. Market Price

Source: Livestock and Meat Statistics
U.S. Dept. of Agriculture
Table 1 Isolation of *M. paratuberculosis* from Wisconsin Cull Cows

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 3 samples negative</td>
<td>189</td>
</tr>
<tr>
<td>Positive: ileocecal valve only</td>
<td>9</td>
</tr>
<tr>
<td>ileocecal valve and cecal contents</td>
<td>2</td>
</tr>
<tr>
<td>valve, node and cecal contents</td>
<td>2</td>
</tr>
<tr>
<td>lymph node only</td>
<td>2</td>
</tr>
<tr>
<td>ileocecal valve and lymph node</td>
<td>1</td>
</tr>
<tr>
<td>lymph node and cecal contents</td>
<td>0</td>
</tr>
<tr>
<td>cecal contents only</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2 Distribution of *M. paratuberculosis* Positive Animals in Markets Sampled

<table>
<thead>
<tr>
<th>Market</th>
<th># Positive Animals/Animals Sampled</th>
<th># of Days Market Represented</th>
<th># Days Market had Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1/72</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>7/30</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0/19</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0/17</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0/14</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>4/11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>0/10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>0/10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>3/6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>private lots</td>
<td>1/16</td>
<td>8*</td>
<td>1</td>
</tr>
</tbody>
</table>

* Total number of private lots
Table 3  Effect of Sample Size and Prevalence when Determining Confidence Intervals

<table>
<thead>
<tr>
<th>If true prevalence detectable for detectable infection would be:</th>
<th>95% Confidence Interval</th>
<th>95% Confidence Interval for true prevalence of infection would be:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p)</td>
<td>( (0.4p)^* )</td>
<td>( \frac{.4p + 2\sqrt{.4p(100-.4p)}}{.4p(100-.4p)/n} )</td>
</tr>
<tr>
<td>for sample size:</td>
<td>for sample size:</td>
<td></td>
</tr>
<tr>
<td>n=100</td>
<td>n=200</td>
<td>n=1000</td>
</tr>
<tr>
<td>5%</td>
<td>2%</td>
<td>0-5%</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0-8</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>3-13</td>
</tr>
</tbody>
</table>

* The sensitivity of the culture methods was estimated to be 40%. Though the sensitivity may be higher for culturing of tissue specimens, the above analysis points out that wider confidence intervals occur when sample size is low.
Table 4  Frequency of Isolation of Salmonellae from Eight Positive Specimens of Pig Feces (adapted from Heard, et al.)

<table>
<thead>
<tr>
<th>Sample order</th>
<th>Specimen Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
</tr>
</tbody>
</table>

Total Positive 5 8 12 1 1 2 2 1
Table 5 Effect of Replicate Culturing on Confidence Intervals

<table>
<thead>
<tr>
<th></th>
<th>If true prevalence of infection is:</th>
<th>Then detectable infection is:</th>
<th>95% Confidence Interval for prevalence of infection would be:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single cultures</strong></td>
<td>5% [1-(.6)(^1)]p=.4p(^*) (\frac{1}{1.4}(.4p\pm\sqrt{.4p(100-.4p)/200}))</td>
<td>2%</td>
<td>0-10%</td>
</tr>
<tr>
<td>of 3 sites</td>
<td>10</td>
<td>4</td>
<td>3-18</td>
</tr>
<tr>
<td>from 200 cows</td>
<td>20</td>
<td>8</td>
<td>10-30</td>
</tr>
<tr>
<td><strong>Duplicate cultures</strong></td>
<td>5% [1-(.6)(^2)]p=.64p(^*) (\frac{1}{1.64}(.64p\pm\sqrt{.64p(100-.64p)/100}))</td>
<td>3%</td>
<td>0-10%</td>
</tr>
<tr>
<td>of 3 sites</td>
<td>10</td>
<td>6</td>
<td>2-18</td>
</tr>
<tr>
<td>from 100 cows</td>
<td>20</td>
<td>13</td>
<td>10-30</td>
</tr>
<tr>
<td><strong>Triplicate cultures</strong></td>
<td>5% [1-(.6)(^3)]p=.78p(^*) (\frac{1}{1.78}(.78p\pm\sqrt{.78p(100-.78p)/75}))</td>
<td>4%</td>
<td>0-12%</td>
</tr>
<tr>
<td>of 3 sites</td>
<td>10</td>
<td>8</td>
<td>2-18</td>
</tr>
<tr>
<td>from 75 cows</td>
<td>20</td>
<td>16</td>
<td>9-31</td>
</tr>
</tbody>
</table>

* Given the culture sensitivity to be 0.4\(^5\), the probability that a positive specimen would result in a negative culture would be 1-0.4 = 0.6. For two cultures done independently from the same positive specimen, the chance of two negative cultures would be \((0.6)^2 = 0.36\); so for two cultures from a positive specimen, the chance of detecting infection would be \([1-(0.6)^2]p\), where \(p\) is the true prevalence of infection. Similar computations were made for triplicate cultures.
PROCEDURES FOR THE FIELD AND LABORATORY PROCESSING OF FECAL SPECIMENS FOR THE ISOLATION OF MYCOBACTERIUM PARATUBERCULOSIS

Diana L. Whipple and Richard S. Merkal

SUMMARY

Paratuberculosis (Johne's disease) is a chronic enteritis of ruminants caused by Mycobacterium paratuberculosis. The most accurate method of identifying infected animals is the cultural isolation of the organism from tissue or fecal specimens. One recommended procedure for the control of the disease is semiannual culturing of fecal specimens from the entire adult herd. This report describes the technique for collecting, transporting, and laboratory processing of the specimens. The identification of M. paratuberculosis isolates is discussed.

These techniques were tested in the field using 43 whole-herd samplings representing 23 herds. Data from this study have been used in some herds to help control the disease by selectivity culling positive animals. The procedures presented are recommended as an aid to control Johne's disease within a herd.

INTRODUCTION

Paratuberculosis (Johne's disease) is a chronic enteritis of ruminants caused by the bacterium Mycobacterium paratuberculosis. Animals usually become infected during the first few days or weeks of life by ingesting feces contaminated with the organisms, which then slowly multiply in the lamina propria and mesenteric lymph nodes. Eventually, the bacteria may be sloughed into the intestinal tract and shed in the feces. Clinical Johne's disease, which is characterized by diarrhea, weight loss, and cachexia, is a terminal syndrome of the infection, which is manifested in adult animals. Although most animals infected with M. paratuberculosis do not develop the clinical disease, other health problems, such as mastitis, lower milk production, and longer calving intervals have been reported.

Paratuberculous animals may be divided into 3 groups: 1) infected, not shedding, no clinical disease 2) infected, shedding, no clinical disease; and 3) infected, shedding, clinical disease. Animals that are shedding the organisms in the feces are the primary source of environmental contamination that may lead to infection of young animals.

Investigators have been working to develop a quick, reliable, and sensitive test to detect paratuberculosis. Although some tests, such as the agar gel immunodiffusion and the enzyme-linked immunosorbent assay seem promising, the definitive diagnosis still is the cultural isolation of M. paratuberculosis from fecal or tissue specimens. Cul-
tivation of the organism requires specialized media containing ferric mycobactin and 8- to 16-weeks of incubation.

Culturing the organisms from fecal specimens has been used extensively to detect paratuberculous animals. One recommended procedure for the control of the disease is semiannual culturing of fecal specimens from the entire adult herd. Once paratuberculous animals are detected, they are to be sold to slaughter.

This report presents the techniques used at the National Animal Disease Center (NADC) for the field and laboratory processing of bovine fecal specimens for the isolation of M. paratuberculosis. These procedures were tested on 2,528 fecal specimens and, in some cases, the results were used to help control the disease by selectively culling infected animals.

MATERIALS AND METHODS

Specimen collection and shipment. Fecal specimens were collected during 1982 to 1984 from 2,528 beef and dairy cattle in 23 Iowa herds, and were used for the present study. Because 13 herds were sampled 2 or more times, there were actually 43 herd samplings processed. Since the present study was to evaluate the method used, the herds sampled were not randomly selected, and the results could not be used to determine prevalence. Participating herd owners and veterinarians contacted the NADC at least one week prior to the desired sample collection time to request a collection "kit." The kit contained numbered one ounce ointment tins, gloves, an identification form, and instructions for sample collection. Most participants requested enough supplies to collect fecal specimens from all cattle that were one year old or older. To reduce fungal contamination of the cultures, participants were instructed to withhold obviously moldy feed from cattle for at least 2 days prior to sample collection. The samples were taken from the rectum using a fresh glove for each animal. The specimens were put into the numbered ointment tins, the animal identification was recorded on the form provided, and the samples and form were sent to the NADC. Specimens were collected and mailed to the laboratory early in the week, so that they would not be held in the post office over the weekend. The samples were shipped without freezing or refrigeration as soon as possible after collection.

Laboratory processing. The specimens were processed in the laboratory as soon as possible after receiving them. About 1 g of feces was placed into a 50 ml centrifuge tube with about 30 ml of distilled water. The samples were shaken in a horizontal position for 15 to 30 minutes; then set in a vertical position and allowed to settle for about 30 minutes. After the heavier particles and debris had settled, 5 ml of the supernatant suspension was removed and transferred to a tube containing 40 to 45 ml of 0.75% hexadecylpyridinium chloride (HPC). The tubes were shaken briefly to mix, and then were allowed to set overnight to decontaminate the samples. The following day, the formed sediment was taken up into a 1 ml pipet and 0.1 ml was used to inoculate each of 4 slants of egg media in 20 × 125 mm screw cap tubes.
Media. The egg media used were egg yolk agar medium (EYM)\(^5\) and modified Lowenstein-Jensen (LJ)\(^4\) containing cycloheximide (0.75 g per liter) chloramphenicol (0.2 g per liter), penicillin (200,000 units per liter), and blue food coloring (7 ml per liter). All media contained ferric mycobactin J or P (2 mg per liter), and 2 slants per sample contained sodium pyruvate (4.1 g per liter).

The slants, with loose caps, were incubated in a horizontal position for one week at 37\(^\circ\) C, to allow the excess fluid to evaporate. Then, the caps were tightened and the slants were set upright for the remainder of the 16-week incubation period.

All slants were examined with the aid of a dissecting microscope. Colonies with morphology and growth rate consistent with \(M. \text{paratuberculosis}\) were subcultured onto one slant of EYM containing mycobactin and one without mycobactin. One tenth ml of a suspension, of organisms in sterile, distilled water (optical density of about 0.01 at 540 nm) was used to inoculate each of the slants for the mycobactin-dependence confirmation test. A smear was made from suspected colonies, and a Ziehl-Neelsen acid-fast stain was done. A colony that grew only on media containing mycobactin and was acid-fast was considered to be \(M. \text{paratuberculosis}\).

Any specimen that yielded at least one colony of \(M. \text{paratuberculosis}\) was considered positive. At least 2 of the 4 slants for any specimen had to be free of growth to be considered a negative test. Specimens with growth other than \(M. \text{paratuberculosis}\) on 3 or 4 slants were discarded as contaminated and reported as no test.

A copy of the identification form with culture results recorded was sent to the appropriate herd owners and veterinarians.

RESULTS

\(Mycobacterium \text{paratuberculosis}\) was isolated from 12 of the 23 different herds submitting specimens and from 22 of the 43 herd samplings processed. The herd size ranged from 2 to 133 animals, and the number of animals shedding the organisms within positive herds ranged from 1.4% to 40%.

DISCUSSION

An effective paratuberculosis control program depends on an early identification and elimination of animals that are shedding \(M. \text{paratuberculosis}\) in the feces.\(^9\) Cultural isolation of the organism from the feces has been demonstrated to be an accurate method to detect these animals.\(^3\) The collection and culture methods used in this study are simple and effective techniques for the isolation of \(M. \text{paratuberculosis}\) from fecal specimens.

To keep the specimens as consistent as possible from herd to herd, collection kits were sent to the participants. We had fewer identification problems when the ointment tins were prenumbered and the animal identification was recorded on the supplied form. We chose to have the
specimens shipped without freezing or refrigeration because a previous study reported that there was no significant loss of viable organisms after 4 days at 23°C.

In the laboratory, the specimens were processed by shaking in water and decontaminating in HPC. This has been the most accurate and reliable technique used to date (unpublished data). Although both the EYM and LJ media were capable of neutralizing the HPC and supporting the growth of *M. paratuberculosis*, the LJ varied more from batch to batch, and was affected more adversely by contamination than the EYM. The LJ frequently was liquefied by contaminants, which made it nearly impossible to detect colonies of *M. paratuberculosis* that may have been growing on a contaminated slant. We have since begun to use only EYM for routine culturing from fecal specimens.

Contamination is a problem encountered frequently when culturing from fecal specimens. Attempts have been made by modifying decontamination procedures and adding agents to the media to control the growth of contaminants. At the NADC, the most difficult contaminants to control are molds and saprophytic mycobacteria. Since mold in the feed has been implicated as a source of contamination, we instructed the participants to withhold obviously moldy feed from the animals for at least 2 days prior to sampling. In some herds, the level of contamination was acceptable (<5%), but in other herds, the contamination was excessive. In one herd, the sample set was processed 3 times, with 100% contamination each time. Most of our contamination problem was due to mold growth; however, for some samples it was due to a saprophytic organism in the distilled water source that was resistant to the HPC. We are now using sterile distilled water to make the decontaminant.

In some herds, the culture results were used to reduce the number of animals shedding by selectively culling positive animals. In one herd the number of animals shedding dropped from 15% to 2.1% in 6 months. In another herd, in which 3 sample sets were processed at 6-month intervals, the number of animals shedding went from 13% to 40%. It was apparent, from the identification forms, that animals that previously had been shedding were not eliminated from the herd.

Although there are problems associated with culturing from fecal specimens, such as prolonged incubation and contamination, it is an effective tool to use to control paratuberculosis. Cultural isolation of *M. paratuberculosis* from feces allows for an accurate identification of animals that are shedding the organisms. Eliminating these animals from the herd will help reduce the rate of paratuberculosis. The procedures used in this study are recommended as an aid to control paratuberculosis within a herd. They are important for the accurate identification and selective elimination of infected animals.

**LITERATURE CITED**

1. Abbas, B., Riemann, H. P, Hird, D. W. Diagnosis of Johne's disease (para-
tuberculosis) in northern California cattle and a note on its economic sig-
2. Buergelt, C. D, Duncan, J. R. D. Age and milk production data of cattle culled
from a dairy herd with paratuberculosis. J Am Vet Med Assoc
3. Chiodini, R. J., Van Kruiningen, H. J., Merkal, R. S. Ruminant para-
tuberculosis (Johne’s disease): The current status and future prospects. Cornell
4. Jorgensen, J. B. An improved medium for culture of Mycobacterium para-
5. Merkal, R. S. Diagnostic methods for detection of paratuberculosis (Johne’s
6. Merkal, R. S., Richards, W. D. Inhibition of fungal growth in the cultural
7. Merkal, R. S., Larsen, A. B., Booth, G. D. Analysis of the effects of inapparent
8. Merkal, R. S., McCullough, W. G., Takayama, K. Mycobactins, the state of the
10. Richards, W. D. Effects of physical and chemical factors on the viability of
11. Sherman, D. M., Markham, J. F., Bates, F. Agar gel immunodiffusion test for
1984;185:179–182.
12. Yokomizo, Y., Yugi, H., Merkal, R. S. A method for avoiding false-positive
reactions in an enzyme-linked immunosorbent assay (ELISA) for the diagnosis
TUBERCULOSIS IN NEW MEXICO
PRELIMINARY REPORT

by
Charles D. Stumpff, DVM, AAVIC, KS
M. A. Essey, DVM, Chief Staff Vet., TB Staff
Ron Pemberton, DVM, VMO, Roswell, NM
R. E. Thompson, DVM, AVIC, Albuquerque, NM

History

During 1985, the El Paso milk shed area experienced an outbreak of Bovine Tuberculosis. The areas covered are portions of the states of New Mexico and Texas. This report will cover only the New Mexico section; however, both areas have many common characteristics. At the present time there is a total of five confirmed herds in New Mexico and five herds in Texas. Many of the herds in both states are closely related.

This particular area is a concentrated dairy area. All herds are maintained in close confinement and range in size 8,000 to 200 head, all classes. The average size herds are approximately 1,000. Most dairies raise part of their replacements and in many cases, part of the growing period is spent in a feed lot. Other replacements are purchased from random sources including dealers in New Mexico and other states. The average cull rate approaches 30% annually. The bulk of the labor force is composed of Mexican Nationals. In general, all infected herds experience a lower sensitivity and lesion rate that one would expect in an environment so ideal for the spread of Bovine Tuberculosis. Many reasons have been advanced for this phenomena but none proven. Some of these are:

1. Low pathogenicity of this particular strain of M. bovis.
2. High cull rate of cows.
3. Dry and mild climate.
4. Increase chronicity of the disease.

Tuberculosis was first disclosed in New Mexico by 6–35 investigations in two separate herds in the spring of 1985. Tuberculosis was later confirmed in both herds from reactors disclosed as result of on-the-farm herd testing.

Sources

Extensive work was performed in determining possible sources. This included a search of brand records in New Mexico. The following sources were identified:

1. Source P. Both of the original herds had purchased animals from this source prior to 1982. Source P also had sold animals into other herds. Herds known to have received cattle from Source P were tested. Two additional herds were found to be infected with bovine Tuberculosis as a result of this trace. Three other herds in Texas were also found to be infected that had received cattle from this source.
2. **Seven Rivers Feedlot, Carlsbad, NM.** Four of the five infected herds, during the past three years, have had heifers in this lot for variable periods of time. Possible exposure could have occurred from across alley contact or by common usage of loading facilities. This feed lot also feeds cattle for slaughter, many are of Mexican origin. This feed lot no longer handles Holstein heifers.

3. **Random Source Purchases.** All infected dairies have purchased replacement animals from dealers in New Mexico and other states; also some purchases have been made from other dairies in the area. One purebred herd disclosed to be infected in the beginning appeared to have no connection to the other herds in the area. However, it was later disclosed that one heifer from this herd was sold to a Holstein heifer raiser as a 4-H calf. This particular heifer raiser bought animals from numerous sources, including one of the current infected herds. This purebred heifer remained in this herd for two years and was resold back into the original herd in New Mexico two years later in 1978. This animal remained in the herd for a period of one year and was slaughtered. Other additions to this purebred herd were twenty years ago. It would appear that the most likely source of the infection in this herd was this particular animal returned in 1978.

Considering the wide movement of animals of random source animals in general, the introduction of a Tuberculosis infected animal from this source would have the potential of eventually causing infection in many herds in this particular area.

4. **Man to Animal Transmission.** In view of the fact that the majority of the labor force is Mexican Nationals, this source cannot be eliminated. For example, skin test results of 300 workers and families at one dairy yielded 49 positive tests. Unfortunately, to the best of my knowledge, no isolation attempts were made. All humans positive to the test were placed on chemotherapy.

**Procedures in New Mexico**

A work force was established at Las Cruces, NM to test all herds associated with Herd P mentioned under sources of infection. All herds adjacent to such herds were also selected for evaluation. One purebred institutional herd was tested at their request. At the time of testing there was no known association with other infected herds in the area. This herd was confirmed positive. Texas has also tested high risk herds with five herds confirmed. Additional herds will be tested that have received animals from known infected herds. This is currently in progress.

Handling of known infected herds created many problems, some of which have not been resolved. Action considered was as follows:

1. **Herd depopulation.** Under present indemnity schedules, depressed farm economy, and indebtedness of the owner, this was not deemed possible. Such action would have not only forced owners into bankruptcy, but, with a domino effect upon lending institutions and other related indus-
tries. The purebred herd felt that they could not sacrifice their established blood lines.

2. In lieu of herd depopulation, the use of the single cervical was recommended. The commercial dairy owners felt that it would have the same effect as procedure number one. The test was recently applied in the purebred herd with 49 R. of 654 animals tested, or a 7% rate. This was less than expected and it may be of help for use of this test in the future in other herds. The State Veterinarian in New Mexico does not oppose single cervical test use, but favors a trial of alternate methods. It is his feeling that he does not wish to force financial ruin upon the industry unless absolutely necessary.

3. Use of the single caudal fold test without the comparative cervical test. This is the procedure currently in use in four of the five infected dairies. Caudal fold responders are classified as reactors. This is also combined with the removal of previous caudal fold positive animals that were classified as negative on the basis of comparative cervical test results. Two herds have been retested, Herd D, 148 R of 7600; Herd M, 1100 animals tested, 8 reactors disclosed. Four animals had compatible lesions from Herd D. Herd M had 8 CFR of 1100 tested. In addition, eleven previous C-C test negatives were classified as reactors. CF positive animals were slaughtered. Three animals were disclosed with gross lesions. One animal was compatible for *M. bovis*.

Procedure 2-3 must be enhanced by good slaughter surveillance of cull animals. There is a hope with quality testing and rapid turnover of animals in these herds, this will eventually lead to eradication. All of these may fail and may eventually lead to Procedure No. 1.

**Current Problems.**

1. Lack of State indemnity to supplement Federal indemnity. Participation by the state in this area would be extremely helpful in initiating procedures for eradication. As a general rule, most states need to improve their effort in the field of indemnity.

2. Lack of understanding of bovine Tuberculosis by cattle owners. Lack of confidence in testing methods by owners. Lack of understanding of the No Gross Lesion animals.

3. Little exonomic loss to owners due to the disease. Eradication procedures are the main cause of economic loss to owners. The disease at its present rate is of no economic consequence. The possibility exists that it may never develop to be an economic problem due to rapid turnover of animals in such herds. In addition, the regulation of not paying indemnity on replacement animals, if they subsequently become reactors creates problems in maintaining cash flow. There is, of course, the public health problem that is associated with bovine Tuberculosis that must be considered.

4. Unreliability of TB test procedures on replacement cattle, i.e., dealer
origin cattle. It is known that a sizable number of TB tests on dealer-type cattle is not reliable.

5. Inadequate slaughter surveillance.

6. Husbandry practices—large number of confined animals in a high density area.

**Future Problems**

This particular TB problem in the New Mexico-Texas area is a serious situation and presents a challenge to our eradication procedures. High density dairy farming appears to be increasing in popularity. The number of animals involved in this situation is impressive; however, compared to the southern California area, it would be relatively small. The consequence of TB introduction in such an area stags the imagination. Other states involved in such operations are Arizona, Florida and others. This current experience may necessitate evaluation of our eradication procedures. Alternate measures may be necessary development to succeed in our goal. No doubt more commitment of funds on the part of the Veterinary Services, State Animal Health Divisions and industry will be required. However, at this particular time, alternate methods should not be considered until established procedures have been fully evaluated.

**Other Epidemiology Consideration**

Complete epidemiology on all herds is still in the development stage. However, currently 259 animals in the purebred herd have been sold since 1981 to 87 premises in Missouri, Oklahoma, Arkansas, Texas and New Mexico that involve the purebred herd. Since the original lists of exposed animals from Herd N, M, it has been determined that infection probably entered the herd in 1978; therefore, this will result in more herds in additional states being evaluated. Additional states receiving animals from infected herds at this time are California, Kentucky, and Indiana.

It is probable that a review of the commercial infected herds will result in many 6-4B cases. There is the possibility that other sources of infection will be disclosed on 6-4A’s. More time will be required to fully develop the epidemiological profile on this particular situation.
PREVALENCE AND ECONOMIC CONSIDERATION OF JOHNE’S DISEASE IN THE NORTHEASTERN U.S.†

by

Whitlock, R. H.,* Hutchinson, L. T.,** Merkal, R.,****
Glickman, L.T.,* Rossiter, C.,** Harmon, S.,** Spencer, P.,* Fetrow, J.,*** Bruce, J.,* Benson, C. E.,* and Dick, J.*****

INTRODUCTION

The Commonwealth of Pennsylvania, Department of Agriculture, has long recognized that Johne’s disease (paratuberculosis) as an important disease of livestock and have made it a reportable disease. The true prevalence of the disease is unknown as no comprehensive survey has been done in the state. The Pennsylvania State Diagnostic Laboratory at Summerdale is currently filled to capacity with requests for Johne’s cultures, which indicates the level of concern the producer and veterinarian have for Johne’s. Since indemnity may be paid for Johne’s fecal culture positive cows by the Pennsylvania Department of Agriculture, Bureau of Animal Industry, the direct cost of Johne’s disease to the state is substantial, i.e., greater than $100,000/year. Johne’s disease is also recognized as a major problem at the national level as indicated by the International Colloquium on Johne’s Disease held at Ames, Iowa, June, 1983.

Paratuberculosis a chronic infective disease of ruminants, has been recognized in the United States for about 75 years. The causative agent is Mycobacterium paratuberculosis, a facultative, intracellular, acid-fast bacterium. Infection with M. paratuberculosis is difficult to control because of a long incubation period, the absence of clinical signs in the early stages, the lack of effective chemotherapeutic agents and the lack of a reliable and practical method for diagnosis.

PROCEDURES

Blood samples, fecal samples and tissues from the ileum, ileocecal lymph node, and rectum were collected from about 1,400 adult Holstein dairy cows at a large slaughter house in northeastern Pennsylvania (Taylor’s Packing Company — Wyalusing, PA). This plant processes more than 200,000 animals per year and accounts for more than 10% of all culled dairy cows in the United States. Three to five days each month a research team visited the slaughter house and collected specimens from approxi-
mately every 12th animal in the processing line for that day. Approximately 120 animals were sampled each month for one year.

The prevalence of Johne’s disease in slaughtered cows was determined by a collective assessment of the culture results from the three specimens obtained from each cow — ileocecal lymph node, ileocecal valve, and feces.

The following data were obtained for each animal sampled at the slaughter house:

1. Live weight
2. State ear tag number(s)
3. Backtag identification number to permit trace back to the auction house where the animal was purchased.

Using the Pennsylvania state ear tag number(s), the Bureau of Animal Industry (BAI) located the herd of origin for each cow. This provided the following information:

1. Owner’s name
2. Address
3. Phone number
4. BAI number

The herd owner was then mailed a detailed questionnaire to obtain the following information:

**HERD INFORMATION**

1. Number of adult cows in the herd
2. Housing for the adult cows
3. Calving area and practices
4. Estimated time cows were left with the calves after birth
5. Calf housing up to weaning
6. Calf feeding practices
7. Heifer housing and management
8. Average herd milk production
9. Previous diagnosis of Johne’s disease in herd

**INDIVIDUAL INFORMATION**

1. Age at slaughter
2. Date of last calving
3. Date culled
4. Duration of lactation (days) at time of culling
5. Reason culled
6. Total lifetime milk production
7. Price received for meat when culled

**RESULTS**

The overall prevalence for Johne’s was 7.2% with 88 of the 1,224 cows sampled over the year long study culture positive. The prevalence on a monthly basis was similar for all months except June when nearly 25% of the cows both from Pennsylvania and from the other states were positive.
The reason for this pattern is unknown.

The sale tag permitted the determination of the state of origin of each cow. The prevalence of Johne's positive cows by state is shown in the following table:

<table>
<thead>
<tr>
<th>State</th>
<th>Number Cows Sampled</th>
<th># Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connecticut</td>
<td>24</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Delaware</td>
<td>4</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Indiana</td>
<td>3</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>25</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>Maryland</td>
<td>58</td>
<td>8</td>
<td>14.0</td>
</tr>
<tr>
<td>Maine</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Minnesota</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Missouri</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>North Carolina</td>
<td>21</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>New Jersey</td>
<td>23</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>New York</td>
<td>311</td>
<td>15</td>
<td>5.0</td>
</tr>
<tr>
<td>Ohio</td>
<td>91</td>
<td>17</td>
<td>19.0</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>502</td>
<td>36</td>
<td>7.2</td>
</tr>
<tr>
<td>Tennessee</td>
<td>2</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Virginia</td>
<td>64</td>
<td>4</td>
<td>6.0</td>
</tr>
<tr>
<td>Vermont</td>
<td>52</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>West Virginia</td>
<td>21</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Ontario</td>
<td>4</td>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>1,224</strong></td>
<td><strong>88</strong></td>
<td><strong>7.2%</strong></td>
</tr>
</tbody>
</table>

The prevalence for states with 50 or more animals sampled are shown below. Compared to all other states pooled together, ninety-five percent confidence limits on prevalence were calculated and an overall chi-square test for uniform prevalence was performed and the results are as follows:

<table>
<thead>
<tr>
<th>State</th>
<th>Number Cows Sampled</th>
<th>Number Cows Positive</th>
<th>% Positive</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania</td>
<td>502</td>
<td>36</td>
<td>7.2%</td>
<td>4.9–9.5%</td>
</tr>
<tr>
<td>Maryland</td>
<td>58</td>
<td>8</td>
<td>13.8%</td>
<td>4.9–22.7%</td>
</tr>
<tr>
<td>New York</td>
<td>311</td>
<td>15</td>
<td>4.8%</td>
<td>2.4–7.2%</td>
</tr>
<tr>
<td>Ohio</td>
<td>91</td>
<td>17</td>
<td>18.7%</td>
<td>10.7–26.7%</td>
</tr>
<tr>
<td>Virginia</td>
<td>64</td>
<td>4</td>
<td>6.3%</td>
<td>0.3–12.2%</td>
</tr>
<tr>
<td>Vermont</td>
<td>52</td>
<td>1</td>
<td>1.9%</td>
<td>0.0–5.7%</td>
</tr>
<tr>
<td>Other</td>
<td>254</td>
<td>12</td>
<td>4.7%</td>
<td>2.1–7.3%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1,078</strong></td>
<td><strong>81</strong></td>
<td><strong>7.2%</strong></td>
<td><strong>5.8–8.7%</strong></td>
</tr>
</tbody>
</table>

\( x^2 = 25.4 \quad P<0.001 \quad \text{Significant difference} \)

The data indicates that Johne's disease is not equally distributed by state. Maryland and Ohio had higher prevalence rates and broader 95%
JOHNE’S DISEASE IN THE NORTHEASTERN U.S. 487

confidence limits for the true prevalence of Johne’s disease than the other states.

The distribution of the study cows within Pennsylvania was compared to the total dairy cow population within the state. For this analysis the state was divided into regions used by the Department of Agriculture, BAI, for administrative purposes. The cow numbers in these regions are known and were compared to the total number of cows sampled from each region. The results were as follows:

<table>
<thead>
<tr>
<th>Region</th>
<th>Total Cows</th>
<th>% of Total</th>
<th>Cows Sampled</th>
<th>% of Total</th>
<th>Sample Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74,300</td>
<td>10</td>
<td>42 - 0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>68,500</td>
<td>9</td>
<td>50 - 0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>100,000</td>
<td>14</td>
<td>87 - 5</td>
<td>18</td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td>58,000</td>
<td>8</td>
<td>38 - 4</td>
<td>8</td>
<td>10.3</td>
</tr>
<tr>
<td>5</td>
<td>107,800</td>
<td>15</td>
<td>53 - 9</td>
<td>11</td>
<td>17.3</td>
</tr>
<tr>
<td>6</td>
<td>244,100</td>
<td>33</td>
<td>163 - 10</td>
<td>33</td>
<td>6.1</td>
</tr>
<tr>
<td>7</td>
<td>81,800</td>
<td>11</td>
<td>58 - 8</td>
<td>12</td>
<td>13.8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>735,000</td>
<td>100</td>
<td>491</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

The cows were not randomly selected from each of the seven regions ($x^2 = 19.62, p < 0.05$). Region 3 which is the location of the slaughter house was over-represented. The % (percent) positive in each region was also not equally distributed. The northern regions had lower prevalence of Johne’s positive cattle than did the southern regions. However, the region with the largest density of cattle — region 6 which includes Lancaster County was not over-represented.

Of the 1,224 dairy cows sampled, 502 (41%) were determined to have originated from Pennsylvania according to the sale tag. For several reasons, including lack of adequate records by the dealer, 35 cows could not be traced to the herd of origin. Of the 467 questionnaires sent out to owners of Pennsylvania traced cows, 385 (82%) were completed and returned. The return rate for the questionnaire return rate of the 36 positive Pennsylvania cows was 89%.

Although there were only 24 non-Holstein cows in the study, no difference in breed prevalence was detected 7.9% vs. 8.3% for non-Holstein cows. The herd size for positive and negative cows was similar:

<table>
<thead>
<tr>
<th>HERD SIZE (ADULT COWS)</th>
<th>Positive Cows</th>
<th>Negative Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>71</td>
<td>73</td>
</tr>
<tr>
<td>Median</td>
<td>57</td>
<td>55</td>
</tr>
</tbody>
</table>

The mean and median ages of positive and negative cows at time of culling were not significantly different. The mean age of positive and negative cows were 5.3 and 5.5 years respectively; the median ages were 4.0 and 5.0 years.
The reasons the farmers gave for culling the cattle were as follows:

<table>
<thead>
<tr>
<th>Reasons to Cull</th>
<th>Positive Cows</th>
<th>Negative Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Low Milk Production</td>
<td>8 – 31%</td>
<td>83 – 27%</td>
</tr>
<tr>
<td>2. Mastitis</td>
<td>6 – 23%</td>
<td>55 – 18%</td>
</tr>
<tr>
<td>3. Infertility</td>
<td>8 – 31%</td>
<td>112 – 36%</td>
</tr>
<tr>
<td>4. Abortion</td>
<td>1 – 4%</td>
<td>8 – 3%</td>
</tr>
<tr>
<td>5. Lameness</td>
<td>1 – 4%</td>
<td>28 – 9%</td>
</tr>
<tr>
<td>6. Poor Body Condition</td>
<td>10 – 40%</td>
<td>30 – 10%</td>
</tr>
<tr>
<td>7. Diarrhea</td>
<td>7 – 27%</td>
<td>6 – 2%</td>
</tr>
<tr>
<td>8. Other</td>
<td>5 – 19%</td>
<td>87 – 28%</td>
</tr>
</tbody>
</table>

The only statistically significant difference between positive and negative cows with the diagnosis of Johne’s disease was for diarrhea and poor body condition. Five, or 19%, of the positive cows were culled for these reasons compared to 3, or 1%, of the negative cattle (p < 0.001).

The cow’s origin was identical for both positive and negative animals, i.e., 77% were home raised while 23% were purchased. Of those 23% purchased animals, about 17% and 19% were from auction, 17% and 22% came from dealers, and 67% and 59% were obtained from other farms for positive and negative cows respectively. The age at purchase was similar for positive and negative animals: 60 and 69% respectively were brought to the farm after calving.

It is not easy to assess management practices with a questionnaire but one attempt to do so was made by the determination of participation in the DHIA program. One might suspect that those herds on DHIA might have better management. The difference in DHIA participation was not significant (40 and 51% respectively), between positive and negative cows.

Inasmuch as many investigators believe the first few weeks of a calf’s life may be critical for exposure to Johne’s disease, questions about nursing and contact time of the calf with the cow were assessed. Nearly 70% of farmers in both groups allowed cows to nurse the calves. However, only 40% of the farmers with positive cows separated their calves before 12 hours compared to 52% of the farmers with negative cows separated cows and calves before 12 hours. During the next six weeks about 55% of the calves in both groups were housed in the same barn as the dam. A similar proportion continued to be housed in the same barn from weaning through six months of age.

The status of the herd of origin concerning Johne’s disease indicated 7 of the 29 (24%) positive cows originated from herds that had been previously determined to have a Johne’s positive cow. Only 11 of the 344 (3%) negative cows originated from known positive herds. The risk of a culled cow having Johne’s disease was 9.6 times greater if she originated from a Johne’s positive herd compared to a herd not known to have Johne’s disease.
An assessment of the economic loss was based on the mean price paid per 100 lbs of body weight, which was $37.24 for the positive animals and $36.32 for the negative animals. The median price was $37.00 for each group. However, the mean total body weight of 1,095 lbs for positive cows was 129 lbs less the mean weight of 1,224 lbs for the negative cows. This difference was statistically significant (p < 0.05).

The mean age (5.8 vs. 5.4 years) of DHIA cows and the mean number of lactations (3.3) was similar for each group. The number of days in milk in the last lactation was nearly identical for each group (268 days). The mean lifetime milk production was lower in the positive cows (46,750 lbs) compared to negative cows (55,390 lbs), but this difference was not statistically significant. The mean herd average for the positive cows compared to negative cows (16,830 lbs versus 15,500 lbs) was higher. Although not statistically significant the mean milk production in the last lactation of the positive cows was 10,130 lbs compared to 13,530 lbs for the negative cows. The difference of 3,400 lbs explains less than half the difference in lifetime milk production (8,640 lbs). This suggested the milk loss attributed to Johne’s disease was spread over the several lactations and was not predominantly attributed to clinical disease in the last lactation. The average daily milk production in the last lactation was statistically different between the groups (39 lbs versus 41 lbs). The difference 12 lbs/day \times 268 \text{ days} = 3,216 \text{ lbs} which correlated well with the last lactation total difference of 3,400 lbs. Assuming an average price of 100 lbs of milk is $12.00; the average milk production loss for a Johne’s positive cow is approximately $386.00 in the last lactation and $1,037 over the lifetime of the cow.

A more comprehensive estimate of the economic loss for Johne’s disease in Pennsylvania was obtained as follows:

1. Total number of dairy cows in Pennsylvania 750,000
2. Cull rate per year 25% 187,500
3. Prevalence of Johne’s at slaughter 7.2%
4. Estimated number of slaughter cows with Johne’s 13,500
5. Milk loss per cow — $386.00 (Last Lactation only) $5.2 million
6. Weight loss per cow 129 lbs 648,000
   $37.00/cwt = $48.00/cow
7. Measured loss — genetic, other diseases; veterinary, etc. ???

Thus, a minimum annual economic loss for Pennsylvania is in excess of $5.8 million per year.

DISCUSSION

The prevalence of Johne’s in Pennsylvania culled cows, 7.2%, is similar to the prevalence recorded in other areas of the United States. A California study found 3.05% positive cultures in 3,140 fecal samples. Wisconsin and Connecticut reported a prevalence of 10.8% and 18% respectively. However these latter studies were based on tissue sample cultures which have a higher sensitivity than do fecal specimens.
Acknowledgement

The Pennsylvania Johne's Research project is a cooperative project involving the School of Veterinary Medicine, University of Pennsylvania; Penn State University; The Bureau of Animal Industry, Pennsylvania Department of Agriculture; and the National Animal Disease Laboratory. The initial project objectives were outlined for three years and was funded by the Commonwealth of Pennsylvania at the recommendation of the State Grange. As the data is evaluated and analyzed, it is anticipated that herds infected with *M. paratuberculosis* can be monitored and recommendations established to facilitate the eradication of Johne's disease from the farms.
IDENTIFICATION BY TRANSFER BLOT OF MYCOBACTERIUM PARATUBERCULOSIS ANTIGENS REACTIVE IN THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Bech-Nielsen, S., Burianek, L. L., Spangler, E., Heider, L. E., Hoffsis, G. F., and Dorn, C. R.

This study describes the characterization of a purified antigen from Mycobacterium paratuberculosis recently made available for serological ELISA diagnosis of paratuberculosis in cattle. This test has 83% and 89% sensitivity and specificity respectively for M. paratuberculosis infection. The protein/polypeptide composition of the purified antigen was compared to that of a crude protoplasmic extract of strain 18 M. paratuberculosis used for the agar gel immunodiffusion test and to sonicated strain 19698 M. paratuberculosis organisms grown on Dorset-Henley synthetic liquid medium. The sonicated M. paratuberculosis contained 27 major proteins/polypeptides, the crude protoplasmic extract 18 and the purified antigen 14 bands using SDS-PAGE electrophoresis analysis.

The antigens of the proteins/polypeptides were identified by the electroimmunotransfer blot technique (EITB). The sonicated M. paratuberculosis revealed 20 antigens (m.w. 34,000–84,000 daltons): the crude protoplasmic extract 3 (m.w. 37,000–45,000 daltons) and the purified extract contained 1 diffuse polypeptide band (m.w. 34,000–38,000 daltons). Selection of M. paratuberculosis antigens reactive in the ELISA will allow us to study their use in the ELISA to improve the sensitivity and specificity of the diagnostic test.

In conclusion we found that 1 diffuse antigen band of the purified M. paratuberculosis antigen are reactive in the serum from a cow naturally infected with M. paratuberculosis.

From the Departments of Veterinary Preventive Medicine, (Bech-Nielsen, Burianek, Spangler, Heider, Dorn), and Clinical Sciences, (Hoffsis), College of Veterinary Medicine, The Ohio State University, 1900 Coffey Road, Columbus, Ohio 43210.

STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM
FISCAL YEAR 1985

Ralph L. Hosker—DVM
Hyattsville, MD

Last year there was a feeling of growing optimism when only three new infected herds were reported out of a total of seven tuberculous herds. This year’s report will cover 30 tuberculous herds. This is the largest number of herds reported since 1976 or in the past 10 years. One, a beef herd, was carried over from the previous year. The total number of herds are made up of 11 diary herds and 19 beef herds. \textit{M. bouis} infection was confirmed in 24 herds and 6 herds were classified as exposed.

If only the total reported herds (30) were considered, the picture would be bleak indeed. However, this is from an estimated total herd population of 1,612,114 in the United States (USDA Statistical Reporting Service (SRS 7-85). Approximately 110 million cattle and calves in the United States are made up of an estimated 77 percent beef animals and 23 percent dairy animals (SRS 7-85).

The total number of tuberculous herds include two outbreak areas. A beef cattle herd detected in North Carolina led to nine additional herd owners in North Carolina and probably all three herds found in Virginia. The second outbreak area is that referred to as the El Paso Milk Shed and is populated by dairy herds in southeastern New Mexico and southeast of El Paso, Texas, where there was 10 dairy premises involved by the end of Fiscal Year 1985. Approximately 28,000 dairy cows are on 33 premises surveyed in New Mexico and 9,500 cattle are on 16 premises surveyed in Texas. The average dairy size is large and many have been developed in the last 10 years. These farms have a high need for replacement animals. Some raise their own replacements and some purchase all of their replacements. Epidemiology indicates that there is probably more than one source of infection for herds in this geographic area.

Four States were added to those that have achieved Tuberculosis Accredited-Free State status while three States previously classified as Free have reverted to Modified Accredited-Free status due to the occurrence of tuberculosis infected herds. There was a net gain of one Accredited-Free State. Since Fiscal Year 1980, tuberculosis infected herds have been found in seven Tuberculosis Accredited-Free States. Three States, Arizona, Vermont, and New York each had one infected herd and therefore did not lose their status. Four of the States lost their status and returned to Modified Accredited-Free. They were Wisconsin in Fiscal Year 1983, North Carolina, Virginia, and New Mexico in Fiscal Year 1985.

An infected dairy herd was found in Ohio; extensive epidemiology and testing has not led to any additional infected herds. There had not been infection in this State since two infected dairy herds were found in Fiscal Year 1980.
An infected beef herd has been detected in Vernon Parish, Louisiana, which is farther north than the herds found in Fiscal Year 1982 and in previous years. As the fiscal year ended, two additional herds were under investigation as the result of slaughter tracebacks to Louisiana.

A group of roping steers in Kansas was tuberculin tested for export to Canada. One out of a group of 15 reacted, and *M. bovis* was later isolated from this subject. Annually 300,000 to 700,000 feeder steers are imported from Mexico after meeting current import requirements. In addition, approximately 2,000 steers that are desirable for rodeo purposes are allowed to be exported by the Mexican Government; the Kansas steers were among animals imported from Mexico.

Two work forces were initiated this year. One was to carry out herd testing of large dairies in the El Paso Milk Shed area; the largest of which contains approximately 8,000 animals. The second work force was to identify and appraise beef cattle on the Island of Molokai, Hawaii, in preparation for depopulation. The largest holding on this Island contains approximately 5,000 animals and was confirmed as infected in Fiscal Year 1983. Two small herds, one infected and one exposed, were found in Fiscal Year 1985. Tuberculosis has been reported from herds on the Island of Molokai since as early as 1940. Efforts to eradicate tuberculosis through testing has not been successful due to rough terrain and difficulty in gathering animals for testing. It was determined in late Fiscal Year 1985 that the most practical solution to the endemic disease situation was to depopulate all cattle on Molokai by sending them to slaughter. In addition to the largest ranch holding, there are approximately 3,500 cattle in approximately 200 smaller herds.

**Figure 1**—In Fiscal Year 1985, four States become Tuberculosis Accredited-Free. They were Oklahoma on October 19, 1984, Pennsylvania on October 24, 1984, Indiana on November 1, 1984, and Kansas on December 6, 1984. Three States had Free status removed and returned to Modified Accredited-Free status. They were North Carolina on March 13, 1985, Virginia on August 1, 1985, and New Mexico on September 1, 1985. There was a net gain of one State for a total of 26 Tuberculosis Accredited-Free States plus the U.S. Virgin Islands. They are as follows: Arizona, Colorado, Connecticut, Delaware, Indiana, Kansas, Maine, Maryland, Massachusetts, Michigan, Minnesota, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, North Dakota, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Utah, Vermont, Wyoming. In addition, the following six states have not had a tuberculosis infected herd in more than 5 years: Alaska, Oregon, Iowa, Missouri, Tennessee, and Georgia.

**Figure 2**—There were a total of 30 tuberculous herds in the United States in Fiscal Year 1985. Of this number, 24 herds were infected and 6 herds were exposed. They were located as follows: Virginia with two infected and one exposed; Ohio with one infected; North Carolina with six infected and four exposed; Louisiana with one infected; Kansas with one
infected; Texas with five infected; New Mexico with six infected, and Hawaii with two infected and one exposed.

It is interesting to note that one of the herd owners with three premises in Virginia, a cattle dealer, had a cow/calf leasing operation that involved 29 premises in Virginia and three in North Carolina in addition to his own three premises with a total of approximately 1,500 animals. One of these leased cattle was found infected with tuberculosis.

The index herd in North Carolina had shipped approximately 193 groups totaling approximately 400 exposed cattle to 16 States. One of the infected dairy herds in New Mexico, a purebred operation, had shipped approximately 285 exposed cattle to 77 owners in 6 States.

**Figure 3**—This pie graph illustrates the means by which the 30 tuberculous herds were found. Tracebacks from regular slaughter directly accounted for nine herds located as follows: Virginia one; Ohio one; North Carolina one; Louisiana one; Texas one; Hawaii two, and New Mexico two. The 14 herds located by tracing exposed animals were all from herds located through slaughter tracebacks. The 14 traced exposed cattle led to confirmed infected herds located as follows: Texas one; Virginia one; New Mexico one; North Carolina five, and to exposed herds located as follows: Hawaii one; Virginia one, and North Carolina four. It can be said that 23 tuberculous herds were found as the result of epidemiologic tracings resulting from regular slaughter sample submissions. Through high risk special area testing in New Mexico and Texas, three infected herds were found in each State. Testing for international movement found an infected group of steers in Kansas. It will be recalled that the last infected herd found through a routine herd test was in Vermont in Fiscal Year 1981. The Kansas steer owner, a supplier of rodeo animals, owned an additional four groups of exposed animals in three States: Oklahoma one; Missouri two, and Illinois one.

**Figure 4**—This figure demonstrates the value of epidemiological tracing both from slaughter (9) and traceout from infected herds (14) a total of 23 such cases. The use of the tuberculin test in special and routine circumstances located the infected steers in Kansas and three dairy herds each in Texas and New Mexico. This total of 30 is the largest number of herds found since 52 herds were found in 1976.

**Figure 5**—Eighteen of 30 known infected and exposed herds were depopulated in Fiscal Year 1985 (60 percent). This figure, while nearly the same (59 percent) as the average figure from 1974 through 1983, is below the average figure of 84 percent for the preceding 5 years and the goal of 95 percent which was proposed in a Tuberculosis Eradication Model developed in 1969.

Actions are being carried out to depopulate the herd on the Island of Molokai as well as approximately 200 other smaller exposed herds. It is anticipated that the confirmed beef herd in Louisiana will be depopulated in Fiscal Year 1986. The group of steers in Kansas will be tested out or sent to slaughter in Fiscal Year 1986. The remaining nine herds that were not
depopulated are large dairy premises found in Fiscal Year 1985. At this time, three of these have been identified in Texas and six in New Mexico where two are under the same ownership.

**Figure 6**—This figure illustrates the proportion of herds depopulated and the States in which they are located. The totals, of course, are the same as the preceding figures which reflect of 30 tuberculous herds 18 were depopulated.

**Figure 7**—These bar graphs illustrate 156 slaughter traceback investigations that were closed in Fiscal Year 1985. They represent 131 feeder cases and 25 cases from adult animals; 15 of which did not have identification. Eleven of the total 156 cases were successfully concluded by identifying herd of origin. The success rate was ten times greater when animal identification was provided with the slaughter sample.

**Figure 8**—This graph uses only figures from Federal slaughter establishments. There was an increase of 463 samples over last year. That is 2,180 samples vs. 1,171 in Fiscal Year 1984. The number of adult animal samples was approximately the same as the previous year; 788 samples vs. 795 samples in Fiscal Year 1984. There was a substantial increase in samples submitted from feeder animals; 922 in Fiscal Year 1984 vs. 1,392 in Fiscal Year 1985. This increase was predictable due to increased importation of Mexican feeder steers in Fiscal Year 1983 (approximately one-half million head). The percent of animals with identification remained about the same for the last 2 years (660 and 672), and there was a 9 percent decrease in the cases from which identification devices were submitted with the samples.

**Figure 9**—The bar graphs in this figure illustrate the number (2,294) of tuberculosis suspicious tissues collected and submitted from regular kill slaughter animals at both State and Federal establishments. The submissions from Federal plants; 2,180 is the greatest number from this source since 1978. This year’s total include 114 submissions from State inspected plants.

Cases are sent to the field for investigation at the time tissues are found to be compatible for mycobacteriosis when examined microscopically or from which bovine tuberculosis is later isolated. This year’s case load of 108 is the largest number of cases since 167 cases were investigated in 1976. Granulomas of unknown etiology were being routinely investigated at that time. The 108 cases represent 90 feeder animals and 18 adult animals.

**Figure 10**—This map shows updated data on bison herds in Fiscal Year 1985. A summary report for Fiscal Years 1984 and 1985 will be given at the Tuberculosis and Johne’s Committee Meeting.

Bovine tuberculosis was found in 1984 in bison herds of the United States for the first time since 1954. The traceback from a lesioned bison bull found on regular kill in April 1984, led to two infected bison herds in South Dakota. Tracing of tuberculosis exposed bison from those herds led
to the discovery of 16 additional infected bison herds by September 30, 1984.

Five additional infected bison herds were found during Fiscal Year 1985. These were located as follows: South Dakota two; and one each in Michigan, Kansas, and Minnesota. In all, a total of 626 exposed bison were traced to 137 owners located in 24 States (4 new States were identified in Fiscal Year 1985). The total of 23 infected herds were located in 10 States as follows: five each in South Dakota and Colorado, three each in Kansas and Minnesota, two in Tennessee, and one each in Michigan, Ohio, North Dakota, Nebraska, and Alabama.

A total of 2,986 bison were slaughtered with indemnity of which 98 were reactors and 1,988 were exposed. All infected bison herds were depopulated except for one zoo display of about six bison. A total of 878 cattle and 42 swine and goats were also depopulated as exposed to tuberculosis. There was no evidence of transmission of tuberculosis from bison to cattle.

In conclusion, the 30 tuberculous cattle herds this year include two outbreak areas involving more than 20 herds. The index beef herd in North Carolina was infected for quite some time before being detected. The first indication that it existed was a slaughter sample from a secondary herd in Virginia.

Exposed cattle from North Carolina had entered at least 16 States. Exposed purebred cattle from an infected New Mexico dairy have entered at least six additional States. Tuberculosis exposed bison entered 24 States or one-half of the country in about 5 years. Bovine tuberculosis continues to be found in imported feeder steers and this year also in rodeo steers from Mexico. The potential for interstate spread of bovine tuberculosis is very real.

Increased herd size, the cost of replacement animals, and the need for farmers to maintain milk base all make depopulation of large dairy herds more difficult to carry out.

States with large dairy operations and purebred beef and dairy herds must consider protection of their herds through import testing and periodic testing of resident herds. There is a need for greater numbers of slaughter surveillance samples to monitor cull animals for beef and dairy herds.

A major work force was activated this past year in Hawaii and in the El Paso Milk Shed area. A large volume of work was accomplished in a short time.

We have made great strides toward the goal of total eradication of bovine tuberculosis in the United States. There is still work to be done and there can be no relenting until this objective is reached.
Tuberculosis Eradication

Bovine Tuberculosis Area Status

September 30, 1985

Accredited Free (26) plus Virgin Islands

Modified Accredited Areas (24) plus Puerto Rico

No M. Bovis for Over 5 Years (6)
Tuberculosis Eradication

Location of 30 Tuberculous Herds

FY 1985
Tuberculosis Eradication

Methods of Locating 30 Tuberculous Herds Initially Detected during FY-85

- Tracing Exposed Cattle from Affected Herds (14)
- Traceback of Regular Kill Slaughter Animals (9)
- Test for Movement (1)
- High-Risk Area Test (6)
Tuberculosis Eradication

Detecting Herds with TB Infection: 1975 through 1985

All Other Tuberculin Testing

Epidemiologic Tracing

1975 76 77 78 79 80 81 82 83 84 85
Tuberculosis Eradication

Herds Found vs. Herds Depopulated

FY 1975-85

1975 76 77 78 79 80 81 82 83 84 85

0 6 8 12 13 17 19 29 30 52
Tuberculosis Eradication
Proportion of Tuberculous Herds Depopulated
FY 1985

[Map showing the proportion of tuberculous herds depopulated in FY 1985 across the United States. The map includes states with numbers indicating the number of herds depopulated and the total number of tuberculous herds.]
Tuberculosis Eradication

Traceback of 156 Tuberculous Cases Closed (Regular Kill Animals) FY 1985

144 Unidentified

96% Unsuccessful

4% Successful

12 Identified

58% Unsuccessful

42% Successful
Tuberculosis Eradication

Number of 6-35's Submitted FY 85
(Federal Establishments)

Number of 6-35's Submitted
Number of Adult Animals
Number of Animals with Identification

Number of ID Devices Submitted


2,180
788 (36%)
529 (67%)
190 (36%)
Tuberculosis Eradication

Tuberculosis Traceback Investigations
Submitted (Regular Kill) FY 1985

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*(State & Federal Submissions)*
Bovine Tuberculosis in Bison—FY 85

* Additional states found with exposed bison—FY 85 (4)

• Infected bison herds detected—FY 85 (5)

□ Accredited—free states involved—FY 84–FY 85 (12)
REPORT OF THE COMMITTEE ON TUBERCULOSIS AND
JOHNE'S DISEASE

Minutes, October 29, 1985

Chairman: S. B. Hurley, Madison, WI
Vice Chairman: V. P. LaBranche, Boston, MA

J. M. Arnoldi, WI; L. R. Barnes, IN; G. Cilley, NH; J. M. Dick, PA; M. A. Essey, MD; J. G. Flint, MN; G. H. Frye, MD; T. J. Hagerty, MN; S. K. Harris, IA; D. E. Hensel, CO; E. M. Himes, IA; A. M. Lewis-Hintz, IA; R. L. Hosker, MD; D. E. Hughes, SD; C. L’Ecuyer, Canada; L. L. Larson, WI; H. Lloyd, FL; A. R. McLaughlin, WI; R. S. Merkal, IA; M. E. Oetting, MO; W. J. Owen, IA; W. L. Searles, TX; M. S. Silberman, GA; D. H. Smith, WA; P. L. Smith, CA; G. R. Snyder, VA; P. L. Spencer, IL; C. D. Stumpf, KS; C. O. Thoen, IA; R. H. Whitlock, PA; B. Widger, NY.

The committee meeting was called to order at 1:30 p.m. on October 29, 1985 at the Marc Plaza in Milwaukee, Wisconsin. There were 86 members and guests in attendance during the two day committee meeting.

1. Dr. Ralph Hosker of the USDA staff, Hyattsville, MD, gave the status report of the State-Federal Tuberculosis Eradication Program. There are currently 30 tuberculosis infected herds in the United States. One of these is a herd disclosed in 1984 and not yet depopulated. Eleven of the 30 are dairy herds, 19 are beef. The complete status report will be included in the proceedings book.

2. Dr. Holly Frisby of the Wisconsin Department of Agriculture, Trade, and Consumer Protection presented a report on a slaughter survey of Wisconsin cull cows to establish the prevalence of infection with M. paratuberculosis. This survey, conducted on 205 animals slaughtered in 1984 and 1985, yielded isolations from at least one of three sites sampled in 16 cows. This infection prevalence rate of 7.8% is comparable to the 10.8% prevalence established in an earlier survey of Wisconsin cull cows. Dr. Frisby discussed the limitations of such a survey and the interpretation of the data. The text of Dr. Frisby’s report will be included in the proceedings book.

3. Dr. Larry Elsken, National Animal Disease Center, Ames, IA, gave a summary of his work on paratuberculosis in goats. Of particular interest was his observation that there is frequently a two to nine month period during which the infected goats shed moderate number of bacteria (M. paratuberculosis) in their fecal material but were negative to all serological tests conducted. The goats did not begin to seroconvert until the time that the number of organisms shed began to increase exponentially and the animals began to show signs of clinical illness. It also appears from Dr. Elsken’s studies that goats as old as six to nine months may be readily infected with M. paratuberculosis.

4. Dr. Evelyn Williams from Iowa State University in Ames reported on the preliminary work she has done in developing and standardizing a PPD
Johnin which can be used for export testing and in other situations where an intradermal test for Johne's disease is required.

5. Dr. Harold McCoy, USDA, Mattoon, IL gave a summary of the epidemiological investigation of the tuberculosis infected herds in Virginia and North Carolina. Information is still quite incomplete due to the tremendous number of traces which will be required due to the involvement of a dealer herd.

6. An overview of the tuberculosis outbreak in New Mexico and Texas was presented by Dr. Charles Stumpff. This report will be included in full in the proceedings book. The political, economic, and biological aspects of the disease in this area of the country have made the situation particularly difficult to deal with.

7. Dr. Hosker and Dr. Mitch Essay also of USDA, APHIS discussed the problem of the introduction of tuberculosis in rodeo steers from Mexico. There are several groups of steers currently under quarantine in the United States as a result of the disclosure of bovine tuberculosis in a steer classified as a suspect as a result of testing for entry into Canada. The consignment from which this animal had originated had been dispersed to rodeo groups and are now quarantined in the states in which they were performing at the time of disclosure. The current owners are reluctant to dispose of the exposed animals without what they feel is appropriate indemnity.

8. Dr. Essey then presented a follow up on the tuberculosis outbreak in bison. Experience has shown that, at least under the conditions of the recent outbreak, bison do not appear to be more susceptible to infection, nor to be less resistant to dissemination of the infection throughout the body tissues. The currently available tests seem to be adequate for detecting infected bison and depopulating infected herds. Dr. Essey's report will be included in the proceedings book.

9. A preliminary report on experimental tuberculosis in bison was given by Dr. Charles Thoen of Iowa State University at Ames.

The meeting was adjourned at 5:15 p.m.

Minutes, October 30, 1985

The second session of the Tuberculosis and Johne's Disease Committee was convened at 1:30 p.m. on October 30, 1985.

1. Several resolutions were passed by the committee and forwarded to the Resolutions committee for consideration. These dealt with the problems of tuberculosis in cattle of Mexican origin, the State-Federal cooperative tuberculosis eradication program, and the handling of bison exposed to or infected with bovine tuberculosis.

2. The Tuberculosis and Johne's disease committee declared by unanimous vote support of joint efforts of the United States and Mexico to eradicate bovine tuberculosis from Mexico. We strongly urge the United States Department of Agriculture to become involved in negotiations toward that end.
3. Dr. Hall from Iowa State University presented results of an evaluation of the blastogenic response of lymphocytes in cattle exposed to *M. paratuberculosis*. A summary of that report will be included in the proceedings book.

4. Dr. Robert Whitlock, University of Pennsylvania, Kennett Square, discussed the findings of the slaughter plant survey conducted in Pennsylvania within the last year. This presentation will be included in the proceedings book in its entirety. In summary, 1216 cull cows were sampled and cultured for *M. paratuberculosis*. Eighty-eight of these samples were positive resulting in a prevalence of 7.2%. Clustering of the positive samples was observed, not only in the prevalence for each state represented in the sample, but within the State of Pennsylvania. Economic considerations were also evaluated using information collected from the herd owners who had shipped cows sampled in the survey.

5. Subcommittee reports

   Embryo transfer from TB infected animals—Dr. A. R. McLaughlin

   The subcommittee recommends that embryos which have an intact zona pellucida and which have been washed at least 10 times with 100 volumes of wash fluid should be used for embryo transfers. This will minimize the transmission of tuberculosis and Johne's disease by embryo transfer.

   Effort should be made to assure that recipient dams are free of tuberculosis and Johne's disease.

   Johne's disease—Dr. R. Merkal

   The possibility of changing the labelling on the Johne's vaccine was discussed. There has been some pressure from some individuals to make the vaccine available for use in herds in which Johne's disease has not been diagnosed. The subcommittee felt strongly that the labelling and use of the vaccine should remain as it is.

   The subcommittee also endorsed the proposed changes in the Code of Federal Regulations which would remove all references to Johne's disease in Part 71 and would delete Part 80. These proposed changes have been published and the public comment period ends November 18, 1985.

   The recommendation was also made that a letter be sent to Dr. Earl Splitter, USDA, CSRS, to request that Johne's disease continue to be listed as a specific priority item for funding by the special competitive grants.

   Resolutions were sent forward from the subcommittee to the full committee for approval. These dealt with Johne's disease staffing within APHIS and the standardization of testing for Johne's disease.

   Uniform Methods and Rules—Dr. R. Hosker

   No changes in the UMR were proposed.

6. Dr. Charles Thoen presented some of the findings of his study evaluating the use of the ELISA test for detecting *M. bovis* infected cattle. Of concern is the observation that a significant number of cattle from which isolations of *M. bovis* are made show no detectable response to the antigens tested.
7. The changes in the Code of Federal Regulations which were discussed in the Johne's subcommittee report were presented in more detail by Dr. Hosker.

8. Dr. L'Ecuyer, Agriculture Canada, spoke briefly about the tuberculosis eradication program in Canada. They have recently identified an infected herd of bison in New Brunswick.

9. Dr. Steen Beck-Nielsen reported on his work to identify *M. paratuberculosis* specific antigens which would be useful in ELISA testing. A summary of Dr. Beck-Nielsen's work will be included in the proceedings.

The meeting was adjourned at 5:30 p.m.
90th ANNUAL MEETING
October 19-24, 1986
EXECUTIVE WEST HOTEL
Louisville, Kentucky

91st ANNUAL MEETING
October 25-30, 1987
HOTEL UTAH
Salt Lake City, Utah

92nd ANNUAL MEETING
October 16-21, 1988
EXCELSIOR HOTEL
Little Rock, Arkansas