General Session

Dr. James Jarrett, Chairman

Johne's Disease (Paratuberculosis) in 1976

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The purpose in presenting this paper is to review recent developments in research on paratuberculosis. Some of the more recent findings that should be of interest to the bovine practitioner are discussed.

Here are two maps showing the results of two surveys on distribution of the disease in the United States, one in 1949 and one in 1971 (Figure 1). Thirty-three states reported presence of the disease in 1949 while 46 states reported it in 1971 (3). Preliminary examination of the maps might indicate that the disease is spreading in the United States. However, this is only a partial explanation. During this period existing diagnostic laboratories have been improved and new laboratories have been built. Culturing techniques have also been improved. Veterinarians are more aware of the disease and are making greater efforts to determine the cause of chronic diarrhea. Therefore, states with widespread distribution may indicate they are showing a greater awareness of the disease rather than more diseased herds than their neighbors.

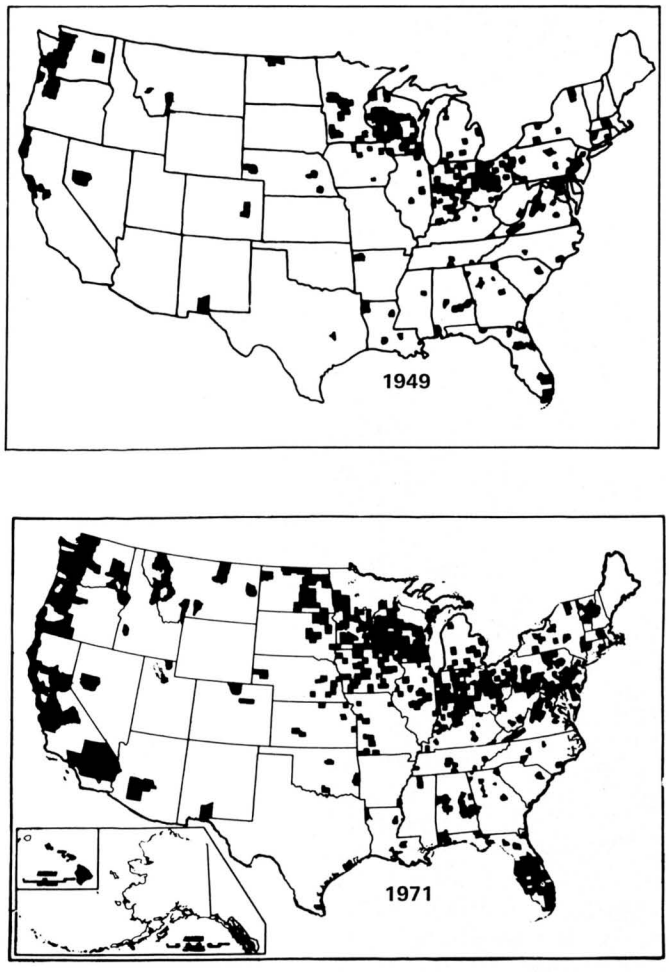

Figure 1. Distribution of bovine paratuberculosis by counties, 1949 and 1971. (Am. J. Vet. Med., 162, (1973), 787.)

A recent study was made of the distribution of the disease in Wisconsin to determine if a relationship existed between infected herds and the soil types on which the herds were located (2). A comparison of the distribution of livestock and the distribution of infected herds showed that the disease did not necessarily occur in the areas of greatest cattle concentration. It appeared that the disease was self-limiting on alkaline calcareous soil and was more difficult to control in herds located on acid soil. Additional studies will be made to confirm these results.

It has been found that economic losses tend to be related to management practices of the herd owners; the poorer the management, the greater the losses (9). An owner of an infected herd can expect to lose 1.5-10% of his adult cattle each year depending, to some extent, on the husbandry he uses. We recently located an infected herd which we used as a source of clinical material. Fecal specimens from 26 adults were cultured and to our surprise 12 were positive. This is the highest percentage of positive culture results we have ever obtained from a herd. During an 18-month period, 14 of 19 heifers between 1-1/2 and 3-1/2 years of age showed clinical signs of disease. These losses are the largest on a percentage basis that we have ever observed.

In another study, it was observed that infertility and mastitis were significantly higher in cattle infected with *Mycobacterium paratuberculosis* than in

normal cattle in the same herd (17). In fact, subclinical losses probably exceed losses due to clinical paratuberculosis alone as much as 75%. Whether this is a manifestation of general lack of resistance to infection by these animals or an indication that paratuberculosis predisposes to the other conditions remains to be determined.

We have found that cattle in infected herds fall into four categories (Table 1): (1) clinically ill cattle, (2) asymptomatic shedders, (3) asymptomatic infected cattle that do not shed enough bacilli to be culturally detectable, and (4) uninfected cattle (6).

Table 1

Categories of Cattle in Herds Affected with Paratuberculosis (Johne's Disease)

Criteria used	Categories 1	2	3	4
Clinical signs present	Yes	No	No	No
M. paratuberculosis cultured from feces	Yes	Yes	No	No
M. paratuberculosis cultured from tissues on necropsy	Yes*	Yes**	Yes**	No
Intravenous johnin test used in diagnosis	Yes	No	No	No

*Microscopic lesions present.
**Microscopic lesions may or may not be found.

Category 1–These are clinically ill cattle usually shedding large numbers of bacilli in their feces. Cattle in this category should have included in their examination an intravenous johnin test (8). We have found this test to be positive in about 80% of the cattle showing clinical signs of disease. However, negative results do not rule out the disease if the animal is showing typical signs.

Recent work has shown that a marked change in the neutrophil-to-lymphocyte ratio occurs in infected cattle following the administration of johnin intravenously (4). This hematologic change may occur in the absence of a temperature increase. This supplemental test is conducted by taking a specimen of blood before and six hours after the johnin injection. A differential leukocyte count is performed on each to determine the change in the ratio of neutrophils to lymphocytes. There has not been enough work in the field to properly evaluate this test as a diagnostic aid.

In addition, a fecal specimen should be sent to a diagnostic laboratory for cultural (which requires 60-90 days) and microscopic examination; in some instances the owner will agree to slaughter for necropsy examination and intestinal tissues can be sent to the laboratory.

It should be pointed out that in addition to the intestinal tract and feces, the bacillus has been isolated from the reproductive tract of both males and females and from the fetus (5,7,14).

Category 2–These cattle may shed bacilli in their feces either constantly or intermittently. Most will eventually develop clinical disease; however, some may be culled from the herd for other reasons before showing signs of disease. Cattle in this category can be detected by culturing the feces for *Mycobacterium paratuberculosis*. They must be shedding at least 100 bacilli per gram of feces to be positive on culture (15). Therefore, an adult must be shedding about one and one-half million bacilli each day to be detected by culturing feces. Positive cultures have been obtained as long as 2-1/2 years before clinical signs were observed. Removing these cattle from the herd reduces the source of infection and results in a higher salvage value than is obtained if clinical signs are present (18).

Category 3–This category consists of cattle from which *M. paratuberculosis* can be cultured from tissues post-mortem, but otherwise appear healthy and are not shedding enough bacilli in the feces to be regularly detected culturally. At present there is no method for diagnosing the infection in these cattle. Many will eventually shed culturable numbers of bacilli in their feces and show clinical signs of disease.

There is evidence that the reason cattle in categories 2 and 3 change to category 1 (clinically ill) has to do with an allergic type antibody-antigen reaction in the intestinal tract which releases diarrhea-producing substances (16). Possibly stress and poor nutrition play a part.

Category 4–Cattle in category 4 are normal, but some may react to skin tests and serologic tests. Possibly some of these cattle have recovered from a light infection.

The number of cattle in each category varies from one herd to another and is probably dependent on husbandry practices (9). In herds in which (1) good sanitary practices are used, and (2) calves are raised separately from mature cattle and receive adequate rations, most cattle will fall into category 4.

In years past, regulatory authorities approved the payment of indemnity for cattle that were slaughtered as reactors to the intradermal johnin test. A five-year study revealed that not all infected cattle reacted to the test, that reactors frequently revert and that hypersensitivity may be intermittent (12). The study showed that the disease could not be eliminated from a herd through application of the skin test. Indemnity is no longer paid for cattle reacting to this test; as a result, the government is saving tax money and the owner of the infected herd is not slaughtering healthy cattle.

Similar studies have revealed that the complement-fixation (CF) test using antigens presently available is not effective for determining infected individuals (13). It was found that the CF titer sometimes changes considerably during a six-month period. It was also found that the titer is slow in developing since marked titers were not observed in most cattle until they were two years old. Yet, many countries importing cattle from the United States have regulations requiring the test. Many exporters

ship by air and they are likely to ship cattle less than two years of age because they weigh less and the freight is less. Therefore, the CF test cannot be depended upon by a purchaser to keep his herd free of paratuberculosis, particularly if he purchased cattle less than two years of age. A history of the herd from which the cattle originate including a negative fecal culture would be of greater value to the buyer than a negative CF test.

It has been reported that an *in vitro* lymphocyte immunostimulation test may be a reliable test for detecting paratuberculosis-infected animals (1). Whole blood is used in this test; since this is perishable, close coordination with the laboratory would be essential. However, this test would require further evaluation and modification before it could be used as a routine diagnostic test.

Removing the shedders from the herd has been found to be an effective method for controlling the disease in a number of herds in Wisconsin (19). Factors that effect the efficiency of this procedure are: (1) the extent of infection in the herd, (2) the period of time the herd has been infected (the longer the period the more difficult it is to eliminate the disease), (3) the size of the herd. (The disease can be eliminated from a small herd in less time than from large herds, probably because small herds are less crowded and there is less opportunity for lateral spread.)

Disadvantages of the procedure are: (1) some infected cattle may be intermittent shedders or not shedding at all, (2) it requires from 60 to 90 days for the bacillus to produce enough growth to form visible colonies (Figure 2).

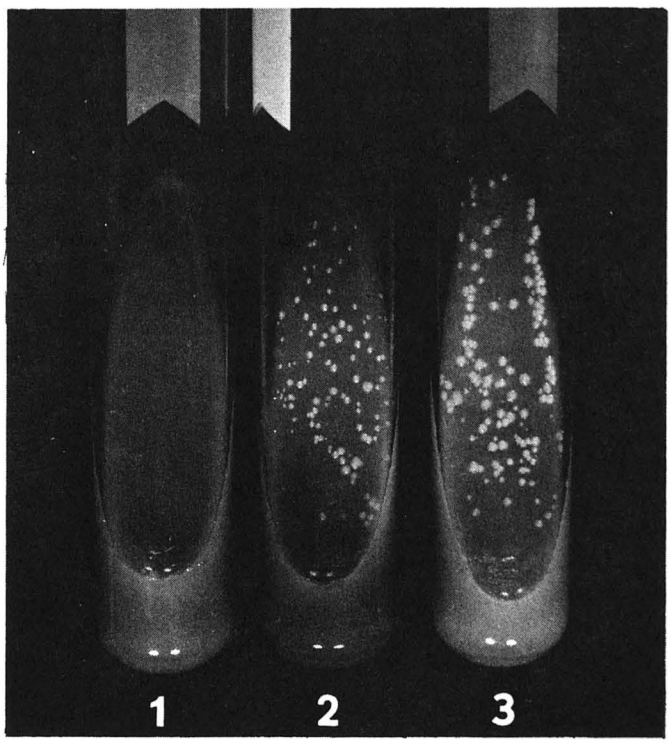

Figure 2. Colonies of *M. paratuberculosis* on culture tubes 2 and 3.

In such a control program it will not be certain that tested herds are free of the disease until those cattle that mingled with *M. paratuberculosis* shedders as calves have been eliminated from the herd through natural attrition.

If reasonable precautions are taken during this period, the disease is not likely to spread to neighboring herds, unless the cattle use the same pasture or come in actual contact with neighboring cattle. It should be emphasized that a shedding animal is a source of infection to all susceptible animals and should be sold only for slaughter. It has also been found that swine can act as a reservoir for the bacillus (11).

It is recognized that animals vaccinated with mycobacterial products develop a degree of immunity (6). Both killed and live products have been used as vaccines. Most work toward developing these products has been done with products for producing immunity to *Mycobacterium tuberculosis* (12). Some work has also been done to develop vaccines against paratuberculosis.

"Vaccinates" become hypersensitive to johnin, avian tuberculin, mammalian tuberculin, and bovine tuberculin. Therefore, regulatory officials have been reluctant to permit field trials with immunogens. We made a study to determine the level of tuberculin sensitivity that would develop in cattle vaccinated with a product prepared from *M. paratuberculosis* and to determine if vaccinated cattle subsequently infected with *Mycobacterium bovis* could be identified by immunologic tests.

Results of comparative tests showed that tuberculosis markedly increased sensitivity of cattle to mammalian tuberculin and slightly increased their sensitivity to johnin (10). Thus, vaccinated cattle exposed to bovine tuberculosis could be identified with comparative tests. On the basis of these findings, several field trials have been started. The largest to date consists of a total of 539 vaccinates and controls. This study has been completed and the results are being compiled for publication.

We are using nonliving *M. paratuberculosis* in all trials because there are several drawbacks to vaccines prepared from living bacilli. Living bacilli may mutate in the host and become virulent and a herd owner who thought his losses from disease increased after experimental vaccination with living bacilli might start litigation to recover damage.

We have several active research projects underway in addition to the vaccine trials. We are studying the bacillus to: (1) develop more specific antigens for diagnostic tests, (2) determine its susceptibility to chemotherapeutic agents, and (3) develop methods to speed up reproduction.

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Discussion

Question: When you have depopulated an area where they have had Johnes disease, how long do you feel is required before you can put animals back into this area?

Answer: This would depend on the premises. If there was a premises that could be well disinfected, with concrete floors and that sort of thing in the barn, and not too many shady areas, it probably would be all right the following spring to put them back. But if the premises would be very hard to disinfect and there are a lot of shady areas that the sun does not get to, then the organism could stay there for a long time. So it depends considerably on the premises.

Question: I'm mainly speaking about coastal areas with range lands, semi-marsh areas, the usual coastal areas.

Answer: I don't think it would be all that big a problem out in the pastures. The primary problem would be around the premises in the yards. If you can do a good job there; for example, if you had a tubercular reactor, it probably would be sufficient.

Question: Using the IV Johnin test, what can we expect as far as false positive and false negatives? (Using the intravenous test?) Yes, using the intravenous test.

Answer: Well, the false positive is most unlikely. Yes, you can have the false negative; we have had false negatives about 20% of the time. But a positive pretty well ties it down–this is Johnes disease.

Question: If we use the sensitivity index along with the thermal reaction, does this increase the sensitivity of the test?

Answer: Yes, this should increase it. If you have both the temperature increase and also the lymphocyte-neutrophil change, I think that it would be almost 100% sure that this is what you are dealing with.

Question: We have depopulated a herd of 116 head of purebred Guernsey cattle in Wisconsin. If it were not that the owner so-called "bit the bullet" and yarded these cattle, we could have put these on the market. We had a high incidence but there were no regulations. There was no reason why this man could not have turned these cattle loose on the marketplace and spread it even further. What suggestions do you have, is there any legislation, and how do you talk a man into this? This man was willing to do this, but there are a lot of them that are not going to do that.

Answer: Yes, there are some guidelines. The U.S. Animal Health Association does have some guidelines along this way. I don't think they are enforced, as far as being a law or anything, but there are guidelines as to what to do in a situation like that. But as far as how many states have real tight legal requirements, I am not familiar with that.

Question: Where can we get the Johnin reagent to test cattle?

Answer: Any state veterinarian can line you up. The federal people do have it, and now I guess there is not a federal office in every state, but the state veterinarian can tell you where the nearest federal office is and you can get it through a federal office because, as far as I know, they do have it on hand.