

SYMPOSIUM: BIOSECURITY AND DISEASE

Johne's Disease: A Hidden Threat

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ABSTRACT

Paratuberculosis, which is also known as Johne's disease, is a chronic, progressive enteric disease of ruminants caused by infection with *Mycobacterium paratuberculosis*. Cattle become infected with *M. paratuberculosis* as calves but often do not develop clinical signs until 2 to 5 yr of age. The clinical disease is characterized by chronic or intermittent diarrhea, emaciation, and death. Although animals with clinical disease are often culled from the herd, animals with subclinical paratuberculosis may cause economic losses because of reduced milk production and poor reproductive performance. Although the economic impact of paratuberculosis on the national cattle industry has not been determined, it is estimated to exceed \$1.5 billion/yr. The diagnosis of subclinical paratuberculosis is difficult. Bacteriologic culture is the most definitive method of diagnosis, but culture is time consuming and labor intensive. Serological assays are not very useful because animals do not develop an antibody response until the clinical stages of disease. Development of assays to measure cell-mediated immunity is critical to accurate detection of paratuberculosis in subclinically infected animals. Although not considered a zoonotic agent, *M. paratuberculosis* has been identified in intestinal biopsy tissue from patients with Crohn's disease, an inflammatory enteritis in humans. Currently, the potential human health risk is being addressed by research evaluating pasteurization of dairy products in the US.

(**Key words:** Johne's disease, ruminants, control)

Abbreviation key: AGID = agar gel immunodiffusion, CF = complement fixation, IFN = interferon, PCR = polymerase chain reaction.

INTRODUCTION

The disorder known as paratuberculosis was first described in 1895 by Johne and Frothingham (23)

who identified organisms in granulomatous lesions in the intestines of affected cattle that stained acid-fast, indicating some type of mycobacterial organism. The organism was cultured from cattle in 1910 and was classified as a mycobacterium by Twort (50) and Twort and Ingram (51). The organism was fully characterized several years later and named *Mycobacterium paratuberculosis*. Paratuberculosis is widely distributed both nationally and internationally in domesticated ruminants such as cattle, sheep, goats, as well as deer, antelope, and bison. The prevalence of the disease in the US is difficult to ascertain because fully comprehensive studies have not been conducted to date. Based upon culture of *M. paratuberculosis* from the ileocecal lymph nodes of culled cattle at slaughter, a study published in 1987 (30) reported that the national prevalence of bovine paratuberculosis in both dairy and beef cattle approached 1.6%. However, major dairy states, such as Pennsylvania, Wisconsin, and California have reported estimates of 7.2, 10.8, and 3.1% infection in culled dairy cows, respectively (1, 5, 57). A Wisconsin study (12) reported an estimated 34% herd infection rate based upon serological diagnosis of paratuberculosis in dairy herds throughout the state. The accuracy of those estimates is limited by the sensitivity of the diagnostic tests used (culture or serologic), accurate recognition and reporting of the disease, and number of animals sampled. It is estimated that losses in the US from paratuberculosis in cattle herds may exceed \$1.5 billion/yr (25). This figure is extrapolated from estimated values of prevalence, computation of financial losses from culling or death of clinically infected cows, and reduced reproductive efficiency, feed efficiency, and decreased milk production in subclinically infected animals. The significance of subclinical infection on economic losses to the producer are detailed in a recent review (24); a reduction in milk production of 15 to 16% accounts for the major portion of net monetary loss (1, 4). Cows that are infected with paratuberculosis beyond second lactation have demonstrated losses of 1300 to 2800 lb (590 to 1270 kg) of milk per lactation (59).

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DISCUSSION

Manifestation of Disease

Cattle become infected with *M. paratuberculosis* as calves but often do not develop clinical signs until 2 to 5 yr of age (29). The primary route of infection is through ingestion of fecal material, milk, or colostrum containing *M. paratuberculosis* microorganisms. Once ingested, *M. paratuberculosis* survive and replicate within macrophages in the wall of the intestine and in regional lymph nodes. After an incubation period of several years, extensive granulomatous inflammation occurs in the terminal small intestine, leading to malabsorption and protein-losing enteropathy. Cattle shed minimal amounts of *M. paratuberculosis* in their feces during the subclinical phase of infection, and yet, over time, this shedding can lead to significant contamination of the environment and an insidious spread of infection throughout the herd. During the clinical phase of infection, fecal shedding of the microorganism is quite high and can exceed 10^{10} organisms/g of feces (8). The terminal clinical stage of disease is characterized by chronic diarrhea, rapid weight loss, diffuse edema, decreased milk production, and infertility. Although transmission of paratuberculosis occurs primarily through the fecal-oral route, *M. paratuberculosis* has also been isolated from reproductive organs of infected males and females. Viable organisms have been isolated from fetuses of infected cows, although intrauterine transmission of *M. paratuberculosis* has not been proven (8).

Diagnosis of paratuberculosis is difficult because of the fastidious growth pattern of the microorganism and because of the paradoxical immune response of the host animal to infection. During the early subclinical stages of infection, the microorganism elicits a cell-mediated response by the host that can be characterized by strong delayed-type IV hypersensitivity reactions, lymphocyte proliferative responses to mitogens, and production of cytokines by stimulated T lymphocytes. As the disease progresses from subclinical to clinical stages, the cell-mediated immune response wanes, and a strong humoral response predominates. The presence of antibody to *M. paratuberculosis* does not protect the host against the disease; indeed, active cell-mediated immunity appears to be essential to keep the infection in check. During the final stages of disease, lack of antigen-specific cell-mediated immune response or complete anergy may result, allowing for rapid dissemination of the infection throughout the host (3).

Diagnosis of Paratuberculosis

Bacteriologic culture is the most definitive method of diagnosis, but culture is time consuming, requiring up to 12 wk of incubation, and also labor intensive (9). Contamination is often a problem when *M. paratuberculosis* is being cultured from fecal specimens, and the National Animal Disease Center has recently incorporated a two-step decontamination procedure to reduce the amount of fungal and bacterial microorganisms significantly (43). Because animals with subclinical disease may shed organisms intermittently in their feces, use of fecal culture alone as a diagnostic method may result in misrepresentation of infection in the herd; only about 50% of *M. paratuberculosis* is detected by fecal culture (39).

Serologic tests for diagnosis of paratuberculosis such as agar gel immunodiffusion (AGID), ELISA, and complement fixation (CF) are relatively easy to perform but are not sensitive (10, 28, 40). The AGID is a simple procedure based upon antigen-antibody precipitation in agar and is most often used as a rapid, diagnostic method for confirmation of clinical paratuberculosis (40). More widely used is ELISA, but usually in conjunction with other diagnostic methods such as fecal culture. Because ELISA is more sensitive than AGID, ELISA can detect subclinical infection more frequently. Estimates for the sensitivity and specificity of ELISA vary widely, depending upon the quality of reagents used in the assay. One major problem with ELISA is the variety of antigens used in the development of these tests, making comparisons between laboratories difficult. Reported sensitivity values for a commercial ELISA kit to detect paratuberculosis antibodies in the sera of cattle range from 15 to 57% for subclinically infected cattle shedding low numbers of organisms in their feces; the average is 88% for clinically infected cattle shedding high numbers of organisms (11, 47). Diagnostic specificity of this commercial kit is estimated to be 99% or greater. Preadsorption of test sera with *Mycobacterium phlei*, an environmental contaminant, prior to testing has markedly enhanced the specificity of the ELISA technique and was a critical step in the development of the commercial kit (62). Development of ELISA tests incorporating more specific antigens to *M. paratuberculosis* has resulted in an increase in the sensitivity of detection (70 to 80%) and a concomitant decrease in specificity (89 to 95%) (2, 52). The CF test is most frequently used to test cattle for import and export purposes. However, as with other serologic tests, the CF test lacks the necessary sensitivity to be used for definitive diagnosis of paratuberculosis infection and has lower specificity than AGID or ELISA.

Recognition that strong cell-mediated immunity in the subclinical stage of infection predominates led to the development of an assay to measure the release of interferon (IFN)- γ by cells that had been stimulated in culture with mycobacterial antigens (60). The amount of IFN- γ released is subsequently quantified in an ELISA using an enzyme-linked monoclonal antibody to bovine IFN- γ . The advantage of this test is its ability to detect subclinically infected animals in a herd, enabling producers to implement more stringent management regimens by segregating infected cattle from the remainder of the herd or by culling them (44).

Methods of detection for paratuberculosis infection have recently been developed using nucleic acid probes combined with polymerase chain reaction (PCR). The first DNA probe that was developed for detection of *M. paratuberculosis* DNA in fecal specimens lacked specificity because the probe also hybridized with DNA from *Mycobacterium avium* (22). More recently, other DNA probes have been developed that are specific for *M. paratuberculosis*. One probe is based upon partial sequence of an insertion element of *M. paratuberculosis*, IS900 (54). A DNA probe test kit based upon the IS900 sequence for diagnosis of *M. paratuberculosis* infection has been developed and licensed for sale in the US (IDEXX, Westbrook, ME). Studies (56) conducted to compare the DNA probe test kit with three different procedures for fecal culture indicate that about 60% of infected cattle detected by fecal culture can be detected using the DNA probe. Therefore, although highly specific for *M. paratuberculosis*, the DNA probe is unable to detect infected cattle that are shedding low numbers of organisms. Another probe that is specific for *M. paratuberculosis* is recombinant clone F57 (33), which is currently being used in some laboratories. A recent modification of the polymerase chain reaction to include two consecutive amplification reactions using nested primers markedly increased the sensitivity of this test (13). This method was able to detect 50 organisms/g of feces compared with 10^4 organisms/g for the commercial kit.

Treatment of Paratuberculosis

Treatment of paratuberculosis with antimicrobials is an expensive venture at best and is ineffective at worst. Standard antituberculosis drugs such as clofazimine, isoniazid, rifabutin, rifampin, and streptomycin have been tested both in vitro and in vivo for effects on *M. paratuberculosis* (46). Although many in vivo studies have demonstrated an improvement of

clinical signs of paratuberculosis in treated animals, the organism could still be detected in the feces. Combination therapy with two or more of the aforementioned drugs has proven to be slightly more effective (15, 34, 41). A 60-d treatment of goats with paratuberculosis that used a combination of streptomycin, rifampicin, and levamisole markedly improved body weight gain, total serum protein, and globulin (15). Fecal shedding of the organism was not observed after treatment, and the organism was not detected in tissues post-mortem. Currently, antimicrobial therapy is not considered a viable option for treatment of paratuberculosis infection because of expense, because of the extended periods that drugs need to be administered, and because of disease recurrence after therapy is discontinued.

Vaccination

Since its introduction in 1926, vaccination has been a controversial method for control of paratuberculosis. Researchers have shown that vaccination with heat-killed or modified live preparations of *M. paratuberculosis* strain 18 has effectively reduced the incidence of clinical disease in dairy herds, sheep, and goats (14, 26, 55). In addition, the number of paratuberculosis organisms shed in the feces of vaccinated animals is reduced, thereby lessening the potential spread to other infected animals in the herd (26, 27). Economic losses from decreased production by infected animals have also been reduced by vaccination (53). However, the benefits of vaccination cannot be clearly distinguished from concomitant improvements in other management practices, such as animal placement, manure disposal, and general hygiene practices. The major disadvantages of a paratuberculosis vaccine include a positive antibody test, which may interfere with serological testing for paratuberculosis or tuberculosis; granulomatous lesions at the site of vaccination; and potential granulomatous reactions from self-injection (8, 42). Use of the vaccine is restricted in the US, thereby limiting the amount of data available on its capacity to prevent infection or the spread of disease. The vaccine has been more widely used in Europe and Latin America with demonstrable benefit.

The advent of new technology should make significant improvements possible to the products that are now available. Several laboratories are currently working with gene products that are specific for *M. paratuberculosis* and that may be feasible for a vaccine. To date, vaccine preparations for paratuberculosis generally comprise whole-cell suspensions or sonicated preparations of the bacterium. There is some

dispute over whether soluble protein preparations are effective for use in a vaccine. A new area of research to be considered is the use of naked DNA from *M. paratuberculosis* as a vaccine candidate. Preliminary studies with other bacterial organisms indicate that DNA vaccination may be an effective way to achieve protection.

Improved Management for Control of Paratuberculosis

One of the foremost considerations for a management program for control of paratuberculosis is proper manure disposal. A common flaw of many dairy operations is the use of the same skid loader for feeding and manure disposal (19). Cross-contamination of the feed is a major contributor to the spread of paratuberculosis, and feces are the major source of the causative organism. Use of improperly treated manure solids to fertilize pastures on the farm is another source of paratuberculosis infection because the organism can survive in the soil for up to a year. Young calves are the most susceptible group of animals in the herd, and calves should be removed from the dam immediately after birth (58). Because *M. paratuberculosis* is shed in the colostrum and milk of clinically infected cows, the caretaker should ensure that calves are fed uncontaminated colostrum and milk replacer to prevent infection. Segregation of infected animals from uninfected animals is a good idea at any age, and the manure of each group should be disposed of separately. Replacement heifers should be kept separate from other members of the herd until their infection status can be ascertained (36). Distinct watering sites must be available for infected and noninfected animals. Stagnant water sources are excellent reservoirs for numerous bacteria, and *M. paratuberculosis* has been found to survive in such water for long periods. The main advice for producers experiencing problems with Johne's disease is to "clean, clean, clean".

M. paratuberculosis: A Human Pathogen?

Recent evidence suggests that the etiological agent in Crohn's disease in humans, a severe inflammatory enteritis involving the terminal ileum, may be of mycobacterial origin (6, 49). Clinical studies have demonstrated the presence of several species of mycobacteria, including *Mycobacterium fortuitum*, *M. avium* ssp. *intracellulare*, *Mycobacterium chelonii*, and *Mycobacterium kansasii* in intestinal biopsy tissues from patients with Crohn's disease (6). More re-

cently, *M. paratuberculosis* has been successfully isolated from patients with Crohn's disease (6, 38). Using the IS900 DNA probe, which is specific for *M. paratuberculosis*, workers (16, 17, 32, 38) have been able to identify the presence of paratuberculosis DNA in intestinal tissue from patients with Crohn's disease. Because the clinical symptoms of Crohn's disease closely mimic those found in animals with Johne's disease, a number of laboratories have proposed that *M. paratuberculosis* may be the causative agent of Crohn's disease (32, 38). However, an equivalent number of studies have been unable to demonstrate the presence of *M. paratuberculosis* DNA in tissue of patients with Crohn's disease (18, 35, 37, 61). Epidemiological evidence correlating exposure to *M. paratuberculosis* with incidence of Crohn's disease is not readily available. However, cows with clinical paratuberculosis do shed viable organisms in their milk at low concentrations (50 cfu/50 ml of milk) (48). In addition, in the United Kingdom, Millar et al. (31) recently demonstrated the presence of *M. paratuberculosis* DNA in milk samples obtained from retail markets. They also described identification of viable *M. paratuberculosis* from the pasteurized retail milk samples after long-term incubation (≤ 40 mo). Interestingly, 9 of 18 milk samples that were previously positive for PCR and 6 of 36 milk samples that were previously negative for PCR were culture positive. Results from that study (31) have been highly controversial and suggest that current pasteurization techniques may not be adequate to kill *M. paratuberculosis* in raw milk. A number of laboratories have recently investigated optimal time and temperature combinations for heat inactivation of *M. paratuberculosis* in milk. Laboratory studies (7, 20) simulating either the standard holding method (63.5°C for 30 min) or the HTST method (71.7°C for 15 s) of pasteurization have demonstrated that a residual population of viable *M. paratuberculosis* survives after treatment of milk (7, 20). However, studies (45) conducted at the National Animal Disease Center using a laboratory-scale pasteurizer have demonstrated that raw milk inoculated with live *M. paratuberculosis* (10^4 or 10^6 cfu/ml) at 72°C for 15 s effectively killed all the bacteria (45). Those data are further corroborated by work (21) conducted in Australia using a small-scale commercial pasteurizing unit. The major difference between the test tube model and the laboratory-scale pasteurizer model was the static versus active flow of milk during heat treatment. Although this difference may not be the only reason for the discrepant results between the two experimental models, results suggest that turbulent flow of milk during the pasteurization process, such

as is achieved in commercial dairies, appears to be necessary to achieve complete inactivation of contaminating bacteria because organisms may clump more readily in a static environment and protect themselves from heat penetration. Although preliminary, those studies indicate that transmission of viable *M. paratuberculosis* from animals to humans via pasteurized dairy products is unlikely and minimizes its potential threat as a zoonotic agent in Crohn's disease.

CONCLUSIONS

There is a need for improved diagnostic tests for the detection of Johne's infection in cattle. Efforts need to be concentrated on the development of simple, rapid, noninvasive tests that can be performed by veterinarians or producers without expensive laboratory equipment. Further research to identify and characterize antigenic proteins that are specific for *M. paratuberculosis* is necessary for improved vaccines. In the interim, improvements in hygienic practices on the farm need to be implemented, and careful consideration needs to be given to manure disposal from infected animals. Further studies also need to be conducted to identify possible sources of *M. paratuberculosis* contamination for humans in the event that this organism is classified as a zoonotic agent.

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