Update on Control and Management of Johne's Disease

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Paratuberculosis or Johne's disease was first described by Johne and Frothingham in 1895.[1] It is a contagious, chronic and usually fatal gastrointestinal tract infection of ruminants caused by *Mycobacterium paratuberculosis*.[1] In cattle, the disease is characterized by a granulomatous thickening of the intestinal wall, leading to progressive emaciation, intermittent to chronic diarrhea and eventual death of clinically affected animals.[1] Some clinically ill animals will also develop a protein losing enteropathy which can lead to the development of edema and ascites.[1]

Prevalence and Cost of the Disease

The disease occurs worldwide and has been reported on every continent.[1] The disease is spreading insidiously and is of great concern to veterinarians and livestock producers.[1] There have been a number of studies done in the United States to determine the extent of *M. paratuberculosis* infections in the American cattle population. In the most recent Wisconsin study, the ileocecal valve, ileocecal lymph node and cecal contents from 205 randomly selected dairy cows were cultured and a (point) prevalence of 7.8% for paratuberculosis was found.[2] A Pennsylvania study found a (point) prevalence of 7.2% when the ileum, ileocecal lymph node and rectum were cultured from 1400 dairy cows from several northeastern states.[3] It is difficult to estimate the severity of paratuberculosis in the United States since each of the abattoir studies differed in the number and amount of tissue samples collected, sample processing protocols and the culture method. A New England study for example, in which six different tissue samples (ileocecal valve, ileocecal lymph node, terminal ileum, liver, tonsil and colon) from 100 randomly selected dairy cows were cultured found a (point) prevalence of 18%.[4] In addition, abattoir studies provide no information on the severity of paratuberculosis in infected herds; the place where test and cull programs are used to control or eradicate paratuberculosis. A recent herd survey in Wisconsin using an absorbed enzyme-linked immunosorbent (ELISA) assay found that 33% of randomly selected herds were positive for paratuberculosis.[5]

A number of studies have shown that fecal culture positive cows (subclinically infected) give roughly 2000 pounds or 15% less milk when compared to their culture negative herdmates indicating subclinical paratuberculosis can have a severe impact on the profitability of a dairy herd.[6] It has recently been estimated that Johne's disease costs the Wisconsin dairy industry a minimum of 100 million dollars annually.[6] If Johne's disease caused only a 1% decrease in the nation's annual milk production of 140 billion pounds, the annual cost to the U.S. would be 150 million dollars (Walker, National Animal Health Monitoring System, personal communication).

The Organism

Paratuberculosis or Johne's disease is caused by *M. paratuberculosis,* a small (0.5 x 1.5 micron), gram-positive, facultative intracellular, acid-fast bacillus.[1] The organism grows in characteristic clumps caused by a network of intercellular filaments.[1] It is identified on artificial media by its characteristic colony morphology (small 1-5mm, firm, glistening, white rough-smooth colonies), acid-fast staining properties and its requirement of exogenous mycobactin for growth.[1] Mycobactin is a cell wall associated iron-binding molecule which is normally produced by many species of mycobacteria and is responsible for intracellular iron storage and/or transport of the molecule. Mycobactin is required for *in vitro* growth of the organism.[1]

Transmission

Paratuberculosis is usually introduced into a herd when healthy but *M. paratuberculosis* infected animals (subclinical infection) are purchased by herd owners. Cattle usually become infected as calves when feces contaminated with *M. paratuberculosis* are ingested, however, adult transmission of the organism has been reported.[1] Other possible modes of transmission of the organism include ingestion of contaminated milk, insemination using contaminated semen, and intra-uterine transmission to bovine fetuses.[1,7] The importance of these alternative routes of transmission in the biology of paratuberculosis is unclear at this time since the or-

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ganism is usually only isolated from milk, semen or fetuses from clinically ill animals, which are usually culled from the herd promptly. Subclinically infected animals that are actively shedding the organism heavily contaminating the calving environment are probably the most important means of transmission of the bacterium. It is well established that animals are most susceptible to infection during the first month of life and that animals become more resistant to infection and development of clinical disease as they mature.[1] This is the reason why most control programs are aimed at breaking the cow to calf transmission of the organism, along with the identification of infected animals for culling from herds.

Pathophysiology

In the host, *M. paratuberculosis* has a predilection for the terminal small intestine where it causes a slowly-developing, granulomatous enteritis. It has been suggested that after ingestion of *M. paratuberculosis* by a calf, the organism is taken up by ileal M cells (found covering Peyer's patches) and later presented to subepithelial and intraepithelial macrophages. Uptake of the organism is enhanced by the presence of antibodies against *M. paratuberculosis*, normally found in the colostrum of serologically positive cows. The reason for infection of the distal ileum may be due to the increased density of Peyer's patches in this region of the gut.[1] The organism slowly replicates in lamina propria macrophages, eventually leading to the formation of multi-nucleated giant cells and spread of the organism to regional lymph nodes.[1] Histologically, early lesions can be missed because granulomas are discrete and scattered, but as the infection progresses, the granulomatous infiltrates coalesce, causing compression and obliteration of the crypts as well as blunting and fusing of the intestinal villi.[1] The lamina propria and submucosa eventually become diffusely infiltrated with macrophages multinucleated giant cells and epithelioid cells which often contain many acid-fast bacteria.[1] The typical gross lesion of paratuberculosis is a diffuse or segmental granulomatous enteritis involving the terminal ileum but in some animals extends throughout the entire gastrointestinal tract.[1] Often in advanced cases there is pronounced intestinal lymphangitis, lymphangiectasia, and lymphadenopathy.[1]

Immunology

The immunologic interactions which occur between *M. paratuberculosis* and its host are complex and incompletely understood. There are several reasons for this lack of knowledge. The first is mycobacterial infections are difficult to study because of the slow, chronic nature of the disease and the complex structure of mycobacteria, in particular the cell wall. Another important reason is the emphasis of Johne's disease research has been directed towards the development of a better diagnostic tests for subclinical infection in cattle and not towards understanding the complex biological relationship between the natural host (ruminants) and the pathogen. Ironically, now that better diagnostic tests have been developed, there is a lack of information on the basic biology of the disease making the development of a control program based on immunological tests difficult.

Cattle usually become infected with *M. paratuberculosis* at a young age and, as such, an understanding of the disease in calves is essential to understanding the biology of paratuberculosis. Calf infection studies have shown that calves can be easily infected by the oral and intravenous routes and will develop lesions characteristic of paratuberculosis and some animals will develop clinical disease. Chiodini[1] has suggested that calves exposed to *M. paratuberculosis* under field conditions first develop a cell-mediated immune response followed by an antibody response. He noted that fecal shedding can commence at any time in naturally infected animals.

The mechanism of host resistance to *M. paratuberculosis* infection is not well understood. As mentioned previously, *M. paratuberculosis* normally resides and multiplies inside host macrophages. This intracellular location of *M. paratuberculosis* coupled with the slow growth of the organism may protect the bacterium from immune system surveillance.[1] These two factors may partially explain why naturally infected animals take so long (weeks to years) to develop a detectable immunological response. Currently, the factors which influence the growth rate of *M. paratuberculosis* in the host macrophage are poorly understood. Zurbrick[8] showed that *M. paratuberculosis* doubled approximately every 7 days when cultured in bovine monocytes and monocyte-derived macrophages. Also, the addition of bovine interferon to monocytes restricted the intracellular growth of the organism.[9] The importance of these *in vitro* findings as it pertains to the pathogenesis of the disease is speculative at this time. Currently, it is generally accepted that young animals that ingest large numbers of *M. paratuberculosis* will develop an immunological response, begin shedding and develop clinical disease much faster than older animals that ingest small numbers of the organism.[1] It is also currently accepted dogma that *M. paratuberculosis* infections in cattle are progressive and animals do not recover from infection.[1]

Developing a Successful Eradication Program

A successful Johne's disease control program re-

quires a dedicated commitment by the livestock owner and his/her practicing veterinarian. Integral to the program is a thorough understanding of the biology of *M. paratuberculosis* infections in cattle as well as a serious effort to break the cycle of fecal-oral transmission in the herd. Therefore, changes are directed at improved sanitation and calf rearing. Fundamental to this is removal of calves from their dams immediately after birth so they can be raised separate from the adult herd until they are at least one year of age. Specific management recommendations are listed in Table 1. In addition to management changes, the herd should be tested for paratuberculosis on a regular basis.

Vaccination

The efficacy of the Johne's disease bacterin is controversial at this time. A recent study in the Netherlands showed that the killed whole cell bacterin did not protect animals from becoming infected with *M. paratuberculosis* but did reduce the incidence of clinical disease.[10] There was no information on the effect of vaccination on fecal shedding or milk production. Based on this information the decision whether to use the vaccine or not must be made on an individual basis depending on the management goals of the producer. It may be indicated in some commercial herds that are primarily interested in the sale of milk and where the owner wishes to control or eliminate *clinical disease* from the herd. It must be emphasized that vaccination alone, without the proper changes in the health management of the herd *will not* control Johne's disease. Our department has observed complete vaccine failure (no decrease in the incidence of clinical disease) in some herds when management changes designed to decrease the risk of exposure or transmission of the organism to newborn animals are not implemented.

Table 1.

1. Calving should occur in clean, dry maternity pens with calves removed immediately after birth.
2. Colostrum should only be milked from a clean dry udder and if possible pasteurized.
3. Raise calves separate from the adult herd for at least one year.
4. Do not spread manure on pastures used for grazing young stock.
5. Avoid exposing calves to drainage from areas where adult cattle are raised.
6. Immediately cull all animals with clinical signs of Johne's disease.
7. Purchase replacement animals from test negative herds for paratuberculosis.
8. Periodically clean and disinfect maternity and calf pens with a phenol based disinfectant.

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Current Laboratory Diagnostics of Johne's Disease

Once the diagnosis of Johne's disease is confirmed in a dairy or beef herd, veterinarians and livestock producers are faced with the dilemma of what to do next. The next logical step is to cost effectively determine the number of infected animals in the herd and begin culling them. In addition, management changes should be implemented immediately to decrease the risk of young calves becoming exposed. You should keep a few simple facts in mind when you are in this situation. First, Johne's disease is caused by a slow growing bacterium called *Mycobacterium paratuberculosis*. This means it usually takes several months or years after an animal becomes infected until it begins shedding detectable amounts of bacteria in the feces and mounts an immu-

nological response. From a practical point of view this means the disease probably has been present in the herd for several years prior to your diagnosis unless the animal was purchased as an adult. Finally no diagnostic test is perfect. A thorough understanding of the sensitivity, specificity, predictive value and cost of each test will help you make a rational decision as to which test or tests you should use to control the disease. Eradication is not easy. Many researchers have shown it takes several years to clean up an infected herd once the owner embarks on an eradication program.[1]

Diagnostic Testing

Every diagnostic test has two fixed intrinsic characteristics; its sensitivity and specificity. Stated another way, these are measures of the rate of true-positive and true-negative results, respectively. Obviously, it is desirable that a test be both highly sensitive and highly specific. Unfortunately this is rarely the case and in fact, the two test characteristics are balanced against each other: as test sensitivity goes up, test specificity goes down.[2] The value of any diagnostic test is best judged in probability terms. Stemming from Baye's Theorem, first published in 1763, the predictive value model calculates the probability that given test result is correct.[3] A key element of the predictive value model is the prior probability of disease (before performing a diagnostic test). In epidemiological terms, we know this as disease prevalence. The predictive value of a positive test (PVP) or predictive value of a negative test (PVN) are calculated using the known test sensitivity (sens) and specificity (spec) together with the disease prevalence (prev) as follows:

$$PVP = \frac{\text{Prevalence x Sensitivity}}{\text{Prevalence x Sens + (1-Prevalence)(1-Spec)}}$$

$$NPV = \frac{\text{(1-Prevalence) x Specificity}}{\text{Prevalence x (1-Sens) + (1-Prev) x Spec}}$$

The impact of prevalence on the predictive value of a test is tremendous. If, for example, 50% of a population has a given disease and a hypothetical test for that disease has a sensitivity of 90% and a specificity of 90%, then 9 out of 10 times either a positive or negative test will be correct in identifying truly diseased or non-diseased in the population. If however, only 10% of a population has the disease, the odds that a positive test result will identify a truly infected individual (PVP) for the same hypothetical animal are no better than a coin toss; 50%. Paratuberculosis prevalence rates in dairy herds range from 0 to over 90%, indicating the need to carefully consider test results when one interprets the results of a whole herd screening test. Generally only tests of extremely high specificity will be suitable for use on animals from populations with very low Johne's disease prevalence, tests of highest sensitivity will be useful over the widest range of disease prevalence rates and each test has its own optimal range of prevalence rates over which it is accurate.

Once the sensitivity and specificity of a diagnostic test have been established, the true prevalence in the herd can be estimated from the apparent prevalence using the standard equation.[4] Apparent prevalence is the number of test positive animals in a herd divided by the total number of animals tested.

$$\text{True prevalence} = \frac{\text{Apparent prevalence + (Specificity -1)}}{\text{Specificity + (Sensitivity - 1)}}$$

Note

The above equation assumes the sensitivity and specificity of the test remains constant in every population of animals tested. This assumption may not always be valid for a chronic disease like bovine paratuberculosis.[5]

Current Diagnostic Tests

The current tests available to diagnose *Mycobacterium paratuberculosis* infections in cattle are conventional fecal culture using Herrold's egg yolk medium, standard complement fixation (CF) test, commercial agar gel immunodiffusion (AGID) test (ImmuCell Corp.), two commercial enzyme-linked immunosorbent assays (ELISA; Allied Laboratories Inc. and Idexx Laboratories) and the commercial DNA probe (Idexx Laboratories). A thorough evaluation of these tests was recently reported by M.T. Collins and myself at the University of Wisconsin-Madison and the results summarized in Table 1.[6,7] We found the CF, AGID and commercial DNA probe worked quite well to diagnose **clinical** Johne's disease but should not be used to diagnose subclinical Johne's disease because of poor test sensitivity. Both ELISA tests were similar in test performance and worked quite well to rapidly estimate the severity of paratuberculosis in individual dairy herds. Currently, Wisconsin is using the Idexx ELISA test, commercial AGID test and conventional fecal culture to run our Johne's disease control program. The AGID test is only used on individual animals to diagnose **clinical** Johne's disease. The Idexx ELISA (sensitivity and specificity of 50% and 99%, respectively) is used initially to screen all the adult animals (≥ 20 months of age) in the herd. The true prevalence of paratuberculosis is estimated from the apparent prevalence. ELISA positive animals are culled as soon as possible unless individual animals are worth a great deal of money. Valuable animals that are ELISA

test positive are immediately fecal cultured.

The question often arises after the initial ELISA test, when should the herd owner test again and which test should be used? The answer is speculative because there is limited information available on how long it takes for infected animals to begin producing detectable amounts of antibody or begin shedding the organism. In other words, if an infected animal is test negative today, how long will it take on average before the animal can be expected to become either ELISA or culture positive. Cost-benefit studies have suggested that unless the true prevalence of paratuberculosis is at least 5%, it is not cost-effective to test for the disease. This decision assumes management changes alone will control the disease in low prevalence herds. Currently in Wisconsin, we recommend herd owners test the entire adult herd once a year except in high prevalence herds (estimated true prevalence ≥ 20%) where the herd owner should test every six months. Tests should alternate between the ELISA test and conventional fecal culture. Conventional fecal culture has a sensitivity and specificity of 50% and 99%, respectively. This recommendation is based on two important observations. The combined test sensitivity of the CSL ELISA and conventional fecal culture is approximately 70% and a number of animals will be positive to only one of the two tests.[8] Alternating tests provides the highest combined test sensitivity. In addition, the reader should remember a culture-based test is desirable for disease control because animals actively shedding the organism in their feces are most infectious to other animals and pose the single largest risk in paratuberculosis infected herds.

The new diagnostic tests for Johne's disease, when used and interpreted properly, will provide us with some important new tools to fight Johne's disease. However, no test will control Johne's disease without proper changes in the health management of the herd. Many of these changes involve improving the hygiene, identifying and removing infected animals and preventing infection of calves with the Johne's disease bacteria.

Table 1. Comparison of seven tests for Johne's disease in adult dairy cattle.

Test	Sensitivity	Specificity	Cost	Speed
RCM	54.4%	100%	$10.00	7 weeks
HEY	45.1%	100%	$4.00	16 weeks
Probe	33.5%	100%	$25.00	2 days
Allied	58.8%	95.4%	$6.00	1 day
IDEXX	43.4%	99.0%	$4.00	1 day
CF*	38.4%	99.0%	$2.00	1 day
AGID	26.6%	100%	$9.00	1 day

RCM = radiometric culture of fecal specimens.
HEY = conventional culture of fecal specimens on Herrold's egg yolk agar.
Probe = commercial DNA probe for *M. paratuberculosis* detection.
Allied= commercial ELISA assay for *M. paratuberculosis* detection.
IDEXX = commercial ELISA assay for *M. paratuberculosis* detection.
CF = standard CF test for *M. paratuberculosis* detection (titer ≥ 1:8 positive).
AGID = commercial AGID test for *M. paratuberculosis* detection.

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