PEER REVIEWED

Johne's Disease Update

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Abstract

Mycobacterium paratuberculosis causes a granulomatous enteritis in cattle that is commonly known as Johne's Disease or paratuberculosis. Young calves acquire the infection *in utero* or within the first few months of age by the fecal-oral route. Available diagnostic tests have limitations, but when used with appropriate management practices, a control/eradication program can be designed to meet the needs of the producer. Control programs are aimed at minimizing or eliminating exposure to the organism. There is no curative treatment for paratuberculosis.

Introduction

This paper will focus on key points pertaining to the organism and the disease, diagnostic aids, and current recommendations for control programs. Johne's Disease or paratuberculosis is caused by *Mycobacterium paratuberculosis* , a gram positive intracellular acid-fast bacillus. The organism is fastidious and requires special media for growth *in vitro* and takes several weeks for results. Because of the organism's slow growth and mycobactin requirement for growth, routine aerobic fecal cultures will not yield the organism. Due to the genetic similarity between *M. paratuberculosis* and *M. avium*, some researchers have recently renamed the microorganism *M. avium* subspecies *paratuberculosis*.[39]

M. paratuberculosis can infect ruminant species such as cattle, sheep, goats, llamas, and deer.

Transmission

Paratuberculosis is generally introduced into naïve cattle herds through purchased additions. Recently purchased animals that appear clinically normal may be shedding large numbers of the organism. Transmission is primarily through the fecal-oral route. Contaminated feedstuffs, fecal soiled bottle nipples or medicators, and fecal contaminated teats are thought to be the most common sources for the fecal-oral route of transmission.

Other potential routes of transmission include *in utero* and colostrum and milk feeding to neonates.[37,44,48,49] Evidence of *in utero* transmission was found by isolation of *M. paratuberculosis* from fetal tissues. In one study, 9 of 34 (26.5%) fetuses were identified as being infected from culture positive cows.[37] In another study, 5 of 58 (8.6%) culture positive fetuses were from dams that were heavy fecal shedders.[49] Investigators have also isolated *M. paratuberculosis* in colostrum (22.2%) and milk (8.3%). The positive samples were from cows that were fecal culture positive.[44] Other investigators identified *M. paratuberculosis* in 9 of 77 (11.7%) milk samples from asymptomatic cows.[48]

Additionally, *M. paratuberculosis* has been isolated from semen and uterine flushings.[22,31,32] In a case report of a clinically normal semen donor bull, *M. paratuberculosis* was isolated from 8 of 31 semen samples over a 21 month period of time. Even though the organism was recovered from the semen, the authors suggested that if appropriate control procedures were instituted at the bull stud, the threat of spread of paratuberculosis was remote. They recommended that purchased bulls come from herds with no history of paratuberculosis. The bulls should be cultured semi-annually. Bulls that culture positive for *M. paratuberculosis* should be isolated from other cattle and any frozen semen collected after the last negative fecal culture should be destroyed.[22]

Embryo transfer has been used to minimize the spread of certain diseases and can be used to export

valuable genetics to other countries. One technique used to reduce or minimize potential pathogens is to wash the embryos after collection. This may not be an effective way to rid the embryos of *M. paratuberculosis.* In a study using bovine ova exposed to *M. paratuberculosis in vitro*, investigators were able to recover the organism from washed ova, suggesting that the organism adheres to the ova during washings.[32] In another study, investigators cultured uterine flushings and recovered the organism from 3 of 4 clinically affected cows.[31] Even though the aforementioned routes of transmission are possible, the fecal-oral route is still considered the primary route of transmission.

Pathogenesis and Clinical Signs

Young calves are considered the most susceptible to infection; however, adult transmission has been reported.[29] Information indicates age-related resistance to infection. The organism gains entry into the host's cells early in life, usually within the first 6 months, and has a predilection for the distal small intestine, especially the ileum. Peyers patches, a concentration of immune cells located in the ileum, are the portal of entry. The organism enters macrophages, replicates intracellularly, and eventually produces a granulomatous enteritis.

A typical history of cattle with paratuberculosis includes intermittent diarrhea that becomes chronic and weight loss despite a good plane of nutrition and a good appetite. The incubation period from infection to development of overt clinical signs takes years. Animals with clinical signs are usually over 2 years of age and many are adults 4-8 years of age. Animals will shed the organism in their feces before exhibiting clinical signs. Hence, once a clinical case is evident, the environment is already contaminated with *M. paratuberculosis.*[45,46]

Prevalence

Early studies using samples taken from slaughter facilities estimated the prevalence of Johne's Disease to be around 1.6% overall (0.8% beef cattle, 2.9% dairy cattle).[24] In the recent NAHMS Dairy '96 study, 79.4% of U.S. dairy cows from 20 states were represented. The study divided the country into the midwest, northeast, southeast, and west regions and herd sizes of less than 50, 50 – 99, 100 – 299, and 300 or more cows. The assay used in this study was an ELISA test with a sensitivity of 45% and a specificity of 99%. There were no statistical differences in adjusted prevalence between region or herd size. The NAHMS results indicated that the total herd prevalence in non-vaccinated herds was 21.6%. This value may underestimate the true prevalence because the study was not designed to detect low prevalence herds.[27]

Another study on Wisconsin dairy cattle using the serum ELISA assay (sensitivity of 50.9%, specificity of 94.9%) found 50% of 158 herds and 7.29% of 4,990 cattle positive. The true prevalence was calculated to be 34% of herds with 4.79% of cattle being serologically positive.[10] Results of a Missouri study using a serum ELISA assay (sensitivity of 43%, specificity of 99%) found that 42 out of 89 (47%) herds and 101 of 1954 (5%) cattle were positive.[52]

Other investigations in the United States have been conducted and included in the Report of the Committee on Johne's Disease in 1993. An ELISA assay was used to determine seroprevalence in Texas dairy and beef cattle. Samples were collected from livestock markets involved in the brucellosis market cattle identification program. The samples were randomly collected from livestock markets for beef cattle. Dairy cattle were sampled at markets that sold dairy cattle exclusively. The findings indicated Johne's was a significant problem in Texas cattle with seropositive results in 25.2% of beef cattle and 13.3% of dairy cattle.[1] Seroprevalence in this report is higher than reported from other areas in the United States. More information needs to be obtained to confirm these findings. Additionally, reported findings of phase 1 of the Johne's Program in Michigan identified 9.1% of Michigan cattle as fecal culture positive at slaughter.[56]

In another report, investigators reviewed epidemiological criteria regarding the associations between soil type (pH) and the prevalence of paratuberculosis. Information gathered in the report indicated little research had been conducted to determine the association between acidic soils and high prevalence of the disease.[19] Investigators conducted a study in Michigan to identify risk factors for herd level infection of paratuberculosis. Their findings indicated that cleaning of calf hutches or pens after each use and application of lime to pastures reduced the risk of herd infection with *M. paratuberculosis.*[18] In a follow up study, the same investigators conducted specific research comparing pH of the soil, iron content of the soil, and application of lime to pastures to the risk of herd infection with *M. paratuberculosis.* The study revealed increased iron content and decreased soil pH were associated with increased incidence of Johne's Disease. Additionally, infections decreased with the application of lime to the pastures.[17] These results could have practical application for control and management of paratuberculosis.

Economic Impact

Obvious losses due to clinical disease include veterinary diagnostic fees, decreased salvage value due to weight loss, potential loss of sales of breeding stock, and death loss.[15] Losses due to subclinical disease include decreased milk production in dairy cattle, increased

culling rate of infected cattle leading to decreased future income, and decreased fertility.[16,23,58] The NAHMS Dairy '96 study addressed the economic implications of Johne's Disease. The study revealed that "reduced milk production was the main factor causing Johne's positive herds to have reduced annual adjusted value of production". During the study, current dollar values were assigned to the financial analysis in order to determine economic losses to producers. The study found "overall, cost per identified Johne's cow, combining both clinical and subclinical infected cows, ranged from $145 to $1,094 per cow with Johne's Disease". In Johne's Disease herds where 10% or more of cull cows were showing clinical signs, the economic loss in the herd was $227 per cow.[27] In a recent study of Michigan dairy herds, there was a 73.5 lb (33.4 kg) decrease in cull cow body weight for each 10 percent increase in cows testing positive for Johne's Disease. The researchers used a commercial ELISA test to determine positive animals.[20]

A study in the northeastern United States sampled cull dairy cows and evaluated cull cow slaughter weight, lifetime milk production, and milk production in the cow's last lactation. Cows with Johne's Disease weighed 129 lb (58.6 kg) less at slaughter, produced 8,637 lb (3,926 kg) less lifetime milk, and produced 3,405 lb (1,548 kg) less milk in their last lactation than negative cull cows. The investigators did not identify any differences between breed, culling age or stage of lactation at the time of culling.[16]

Another study of the economic impact of Johne's Disease on production was conducted in a 210 cow dairy herd in New York. Researchers found that in second lactation and older cows in milk greater than 100 days, cows positive for Johne's Disease had lower mature equivalent milk production and were culled at a faster rate than were cows testing negative for Johne's Disease. Interestingly, Johne's positive cows had significantly lower rates of new and chronic mastitis infections than did Johne's negative cows. The reduced cases of mastitis may have been due to *M. paratuberculosis* acting as an immunostimulant.[57,58]

Diagnosis

Johne's Disease is reportable in many states, however, the consequences for failure to report the disease vary from state to state. Currently, there is no reliable test that can detect early infections prior to shedding the organism. Due to its high specificity, fecal culture is considered the "Gold Standard" for diagnostic tests in animals shedding the organism at the time of sampling. However, if the animal is not shedding the organism at the time of sampling, or the animal is shedding only a few organisms, the test may be negative and not identify the true status of the animal. The accuracy of this test is limited by its sensitivity. The sensitivity of the fecal culture is approximately 50% for intermittent shedders shedding less than 10 organisms per gram of feces. A disadvantage of fecal culturing is the time required for results. Turn-around time from sample submission to completion is 8-16 weeks.

Recently, a probe has been developed to detect *M. paratuberculosis* DNA in the feces of infected animals. It has the advantage of fast turn-around time, usually a few days, and relatively high specificity (97-100%).[40,54] Similar to other tests described above, the probe is dependent on the animal shedding the organism in the feces. It does not detect subclinical infections with the same sensitivity as fecal culture.[54,59] The test also requires special equipment and additional training for laboratory personnel, and is relatively expensive to perform. It does offer the practitioner a test with high specificity to confirm a clinical case of Johne's Disease.

At present there are 3 commonly used serum tests; the complement fixation (CF), agar gel immunodiffusion (AGID), and enzyme-linked immunosorbent assay (ELISA) tests. These tests all have a relatively short turn-around time from sample submission to results; however, all of these tests are also limited by their sensitivity. As the prevalence in a given herd increases, more animals will become shedders and the sensitivity (or the ability to correctly identify true negative animals) of these tests will increase. This fact is supported by comparative data that showed a significant increase in sensitivity of these assays between animals that were shedders as compared to non-shedders.[41] Although these serological assays have limitations, they are an integral part of a successful control/eradication program when used in conjunction with management changes.

Foreign countries are concerned about importing cattle with paratuberculosis. Most countries use the CF test as their standard test for importation testing. When the CF test is positive, the animal is likely shedding many organisms in the feces. Unfortunately, the test does not detect subclinically infected animals.[7,29,55] Different investigators have evaluated the sensitivity and specificity of the CF test. When the CF test was compared to the AGID, ELISA, and fecal culture, investigators found only an intermediate level of sensitivity.[5] Other investigators have used a titer of greater than or equal to 1:8 to determine positive animals and found the sensitivity of the CF test to be comparable to a commercial ELISA test.[41]

The AGID test is considered highly specific (99-100%), but unfortunately sensitivity is low (25-50%).[7,29,39,41,55] This test is commonly used when animals have clinical signs of Johne's Disease and the practitioner needs additional support for the diagnosis. The low sensitivity of the AGID test may produce false negative results when an animal may actually

be infected. Because of low sensitivity, the AGID test is not considered a practical assay for screening a herd to determine prevalence of paratuberculosis.

The ELISA test has advantages similar to the other serologic assays, which includes ease of sampling (serum), short turn-around time, and high specificity (99-100%).[7,30,39,41,47,55] As with other highly specific assays, a positive test result is considered fairly reliable. On the other hand, the test has a sensitivity of approximately 50%. When used in conjunction with management changes and other tests, such as fecal culture or DNA probe, the ELISA test is considered a good herd screening test. Several control and eradication programs recommend the ELISA as the initial herd screening test.[7] To further confirm individual cases, a fecal culture is generally recommended.

In an individual animal suspected of having paratuberculosis, biopsy of the ileum, retrieval of a mesenteric lymph node in the ileocecal area, and demonstration of acid-fast organisms in the tissues helps confirm the diagnosis. The organism can be identified by acid-fast staining of impression smears or histopathology. A less invasive procedure is a rectal mucosal biopsy.[29] Since the organism has a predilection for the distal small intestine, positive rectal mucosal specimens generally occur later in the course of the disease.

Management and Control

Management of this disease can be challenging for both the veterinarian and the producer. Currently, there is no known curative treatment for Johne's Disease, and the palliative treatments are cost prohibitive. Infected animals will eventually die. Recently, the National Johne's Working Group has developed workbooks for beef and dairy herds to aid veterinarians. This information has been provided to state veterinarians and printed in *The Bovine Practitioner*.[14,20,34,35] This paper highlights methods of herd management and refers interested readers to more detailed information in referenced articles or to their state veterinarian.[6,14,27,51]

Economics dictate many production management practices. Practitioners should gain as much understanding about the producer's business as possible and become familiar with their management practices and capabilities before a control program is instituted. Considerations before a program is outlined include determining the primary business objective of the producer, the length of time the producer plans on staying in business, and the willingness of the producer to engage in a control program.[11] It may be difficult to develop an effective program for farms that have a high turnover of employees.

The veterinarian's knowledge of the disease, the proper use and interpretation of diagnostic tests, and familiarity with the producer's management program will help in the decision regarding specific herd control programs.

One of the first steps in developing a control program is to perform a risk assessment for Johne's Disease in the herd. Then develop a management plan for control and prevention. At some point it is advisable to determine the infection rate in the herd. To estimate the true prevalence in a population, one must know the sensitivity and specificity of the tests being used and the apparent prevalence in the herd. The formula to calculate true prevalence is as follows where: AP = apparent prevalence, Se = sensitivity of test used, and Sp = specificity of the test used.[36,38]

$$\frac{AP + (Sp - 1)}{Sp + (Se - 1)}$$

For example, using a test with a 50% sensitivity and a 99% specificity would give an estimate of the true prevalence in a herd as being twice the apparent prevalence. The AP is defined as the number of positive samples divided by the number tested. Apparent prevalence is usually obtained by testing all cattle in the herd that are one to two years of age and older.

Once the prevalence has been determined and management practices have been surveyed, a specific program can be outlined consistent with the economic capabilities of the owner. Clients can easily be overwhelmed with recommendations. In the most recent NAHMS survey, only 55% of dairy producers were familiar with the disease.[27,53] Therefore, in order for the control program to be effective, the client must understand the facts about the disease, the potential economic impact, and the goals of a control program. Follow up visits will be required to monitor progress of the control program.[6,14,33]

Control program recommendations are based on minimizing or eliminating the exposure to the organism.[14] Calves should be born in a clean environment (calving pen or pasture). Dairy calves must be removed from the cow immediately after birth to eliminate the potential exposure to fecal soiled teats. Newborn calves require an adequate quantity of good quality colostrum. The colostrum must come from test negative cows. Producers should not pool colostrum from different cows or feed discarded milk to their calves. In infected dairy herds, calves should be given high quality milk replacer.[27,39]

The above recommendations are not practical for beef producers. Management suggestions for beef herds have been outlined in *The Bovine Practitioner*.[34] Whenever possible, the udder and belly of the dam should be cleaned to minimize fecal contamination of the teats. Calving pens should have adequate bedding and be cleaned after each use. Pens should be for single animal use and calving purposes only. No sick animals should be housed

in these pens. If dams calve outside, the pairs should be removed to pasture immediately after the cow and calf have bonded. Manure and mud accumulation should be at a minimum in these outside areas.[34]

Additional recommendations to minimize exposure of the suckling calf to the organism include measures to prevent environmental contamination by manure from suspected cows. Feed and water should be free of fecal contamination.[34]

When possible young replacement stock should be separated from the adult herd for their first 6-12 months of life. Replacement animals must not be allowed contact with feedstuffs or water that may be contaminated with fecal material from the adult herd. Equipment that comes in contact with or is used for manure handling must not be used to transport or mix feedstuffs. Manure should not be spread on pastures where young cattle graze. Any adult animal that exhibits clinical signs of paratuberculosis must be separated or culled immediately to minimize the amount of shedding of infectious organisms into the environment.[27,39]

Ideally, if a producer is purchasing animals, they should come from herds which have been tested negative for Johne's Disease. Mature additions should be tested negative prior to inclusion into the main herd. Veterinarians should recommend that producers not purchase any cattle from herds with positive Johne's status unless a risk assessment determines that the herd is at very low risk for Johne's Disease.

These measures may be difficult to achieve in all situations, especially in beef herds. After a control/eradication program is instituted, reviewing the progress of the program will allow adjustments as required.

Recently, the U.S. Animal Health Association has approved a new voluntary Johne's Disease herd status program for cattle. The program encourages producers to work with an accredited veterinarian and enroll in the program. There are 2 tracks, standard and fast. The choice of tracks depends on how aggressively a producer wants to identify and remove the Johne's Disease risk from the herd. There are 4 different levels a herd may attain, level 1 through level 4. The higher the level (level 4) a herd attains in the program, the less the risk of infection with paratuberculosis. The program uses ELISA tests and fecal cultures to identify positive animals.[26]

Zoonotic Potential

Through television, newspaper or magazine articles, personal experiences, and the internet, the public has become more aware of zoonotic diseases. Crohn's Disease in humans is a chronic, progressive disease causing a granulomatous ileocolitis. Patients undergo long term steroid therapy and often require surgical intervention to control the disease. The etiology of Crohn's Disease has not been definitively determined. In 1984 researchers isolated *Mycobacterium* sp. from some Crohn's patients.[4] Much interest and research regarding the causative agent of Crohn's Disease has occurred since that time, but researchers from around the world have had variable success in reproducing the results.[21]

With the use of an IS900 polymerase chain reaction (PCR) test, investigators have identified *M. paratuberculosis* organisms in milk from retail outlets in England and Wales.[25] This has raised questions about the relationship between Johne's Disease and Crohn's Disease. Researchers inoculated milk with various concentrations (colony forming units [cfu]/mL) of *M. paratuberculosis* and simulated pasteurization under laboratory conditions. Viable organisms survived the simulated pasteurization techniques used in the laboratories.[2,12] Researchers from Wisconsin compared thermal death curves of some human and bovine strains of *M. paratuberculosis* and showed that if the concentration of the organism was greater than 10^1 organisms/mL in milk, it may survive high temperature, short time (HTST) pasteurization.[50] Other investigators inoculated raw milk with 10^6 cfu/mL of *M. paratuberculosis* organisms and found viable organisms present in the milk samples when heated to 194° F (90°C) for 15 seconds, but a longer holding time of 25 seconds at 161.6° F (72°C) inactivated the organism.[13]

It is unknown however, if the organism can survive current commercial pasteurization techniques used for milk. Recently, scientists compared the results of inactivation of *M. paratuberculosis* using the holder test tube method and a laboratory scale pasteurizer unit. Viable bacteria were isolated using various temperatures and times for the holder test tube method, but no viable organisms were detected using the laboratory scale pasteurizer. They concluded that transmission of *M. paratuberculosis* was unlikely to occur in pasteurized dairy products.[43]

Public health researchers from The Netherlands developed a modeling approach to determine human exposure through pasteurized milk. Specific data regarding the amount of organisms shed by clinical and subclinical cows is minimal, therefore, their model incorporated estimates. The model suggested that consumers may be exposed to 0.5 cfu/liter of pasteurized milk. This contamination is likely due to milk from clinically affected cows.[28]

Researchers have questioned whether pasteurization of milk will effectively destroy the organisms while they are residing in macrophages. To address this concern, researchers infected bovine mammary gland macrophages with *M. paratuberculosis* and used a laboratory scale pasteurizer unit to heat treat the milk samples at 161.6° F (72°C) for 15 seconds. No organisms were cultured. The researchers concluded that the organism

inside the macrophage did not survive pasteurization.[42] With current concerns about food safety, confirmation of a link between *M. paratuberculosis* infected cattle and Crohn's Disease could have a tremendous impact on both beef and dairy cattle industries.[3,8,9]

Information pertaining to Johne's Disease can be found on internet websites. Suggested websites that the practitioner may find useful include:

http://www.vetmed.wisc.edu/pbs/johnes/ipminfo.html
http://www.vetmed.wisc.edu/pbs/johnes/index.html
http://www.byc.com.au/paratb
http://www.usaha.org/njwg/jdplan.html

Summary

Johne's Disease presents a challenge to the veterinarian from both the diagnostic and client education perspectives. Paratuberculosis may be more prevalent in all major dairy regions of the United States. Available diagnostic tests have limitations, but with a better understanding of these limitations, a practical approach to control and eradication of the disease is possible. Management practices are aimed at minimizing or eliminating exposure of the animal to the organism. If the link between Crohn's Disease and *M. paratuberculosis* is found, it could have a significant impact on livestock production.

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Abstract

Dietary protein and the reproductive performance of cows

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Increasing a cow's intake of dietary protein intake can increase its milk production, but can also reduce its fertility. This paper reviews the effects of increasing dietary protein on the fertility of the dairy cow, and the mechanisms that may produce them. The effects vary widely, but all stages of the reproductive cycle from the return to cyclicity after parturition, to the survival of the embryo, may be affected. However, the underlying cause of the link between protein intake and fertility is unclear. Fertility could be reduced by a direct toxic effect of protein breakdown products, but alternatively the increased energy demand for their metabolism could be responsible. The effect of protein degradability is also uncertain. Excess rumen degradable protein is commonly associated with reduced fertility, but similar effects are produced by diets that contain excess rumen undegradable protein. Increasing the intake of protein of all degradabilities has significantly different effects on blood biochemistry than a reduction in the intake of energy, suggesting that not all the effects of protein are due to energy imbalance. The primary site of action of the effect is also unclear. Limited evidence suggests that it is localised to the reproductive system, but effects on the pituitary and hypothalamus, as well as the ovary and uterus, have all been postulated. It is also uncertain what toxic principle is involved. Ammonia, nitrate and urea have all been suggested, but there is no conclusive evidence. Although a high protein intake has been postulated to have an effect on fertility for over 30 years, the evidence remains inconclusive, and the aetiology and pathogenesis of the effect remain obscure.