MARCH 2001
Volume 1, Issue 1

Large Animal VETERINARY Rounds

AS PRESENTED IN THE ROUNDS OF THE DEPARTMENT OF LARGE ANIMAL CLINICAL SCIENCES OF THE WESTERN COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF SASKATCHEWAN

Managing Johne's Disease in a Beef Cow Calf Herd

by Sylvia Checkley, DVM; John Campbell, DVM, DVSc; Eugene Janzen, DVM, MVSc

Heightened awareness of Johne's disease due to public health concerns has brought management and control issues to the forefront in veterinary medicine. This disease also has far-reaching significance related to herd economics and international trade. The practical and commonly used tests for diagnosis and their important features will be discussed. Management recommendations for control of Johne's disease in a cow-calf herd are presented in this issue.

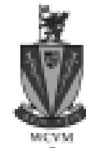
A herd example

The owner of a commercial herd of 300 cow calf pairs reported that over the past 10 years there were a few cases of mature cow chronic diarrhea annually that was unresponsive to treatment. In 1997, two young cows (2- and 3-years-old), were admitted to the Large Animal Clinic of the Western College of Veterinary Medicine. The cows showed symptoms of chronic diarrhea and emaciation, yet were still bright, nonfebrile, eating, and drinking. A tentative clinical diagnosis of Johne's disease was made.

These cows were euthanized and necropsies were performed. Gross lesions included granulomatous ileitis and lymphadenitis. These are the classical lesions of Johne's disease, but they are not always present, making gross signs alone inadequate for diagnosis. The definitive diagnosis of Johne's disease was based on the demonstration of acid-fast bacteria in the macrophages of ileal tissues in smears and histology.

After the first definitive cases of Johne's disease were established, a portion of the herd was bled for Johne's testing. An ELISA test for antibodies against the Johne's disease organism was used as a screening test. AGID was used as a confirmatory test for cows that were doubtful on the basis of ELISA (Table 1). A total of 13 animals were positive out of 103 animals tested. At the time, there were 5 clinically ill animals. Fecal samples were submitted for culture. The five fecal cultures taken from the clinically ill animals were positive for *Mycobacterium avium* subsp. *paratuberculosis*. These results indicated that an endemic problem in the herd was starting to become a major problem. The herd prevalence was 13%. A larger number of the younger animals were affected, but there were a few cases in the older animals (Figure 1). Some new animals had been introduced into the herd about 10 years previously and these may have been the source of infection.

An ongoing longitudinal study is in place for this herd. The plan for the year 2000 included measuring the herd prevalence rate by ELISA and testing all animals that were 2 years or older. Confirmation of diagnosis with fecal culture was important to the owner.



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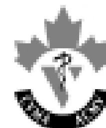

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TABLE 1: Summary of tests used in Johne's disease control programs

Test	Sample	Time required	Cost	Accuracy
Fecal Culture	Feces	6-16 weeks	Expensive	Definitive
ELISA	Serum	24 hours	Inexpensive	Variable
AGID	Serum	24 hours	Inexpensive	Good on clinical cases

Because of the rapidity with which ELISA testing can be performed, it was possible to collect and hold feces for culture on the positive and suspicious cows identified by the ELISA sample to positive ratios (more about this later).

Discussion

Veterinarians are exposed daily to incidents associated with Johne's disease that may lead to disease investigation and the request for control programs. These threshold incidents include:

- Individual animal definitive cases presented to the clinic or the veterinarian.
- Export testing for producers. Some countries,like Japan, require individual animal ELISA and fecal cultures before allowing animals to be exported. This will likely expand in the future to include whole herd testing.
- Public pressure regarding food and ground water safety, as concern about the possible link between Johne's disease and Crohn's disease increases.
- Screening programs to detect seroprevalence of certain diseases.These are commonly carried out by various government agencies.A plan of action may be required for Johne's positive animals.
- Herd certification for producers who wish to sell breeding animals.This will need to be based on herd tests, as individual tests are not accurate for young animals.
- Pressure by producers who are concerned about issues of biosecurity (ie, animals going onto community pasture).

Investigating and managing this type of problem requires knowledge of a number of different areas. We will review the natural history of the disease and examine the diagnostic tests,their accuracy, and application.

Natural history of Johne's disease

The causative organism of Johne's disease was renamed in recent years as *Mycobacterium avium,* subsp. *paratuberculosis,* but it is still commonly known and referred to as *M. paratuberculosis*. This organism is quite resistant;its rough waxy cell wall is responsible for resistance to environmental factors and the Mycobacterium is known to survive in the environment for over a year. Drying, sunlight, and alkaline soils decrease survival of the organism.The intracellular infection and slow progression of disease makes the organism difficult to detect on screening tests.Transmission is via the fecal-oral route with large numbers of organisms spread in the feces of

FIGURE 1: Age distribution of Johne's positive animals in the infected herd.

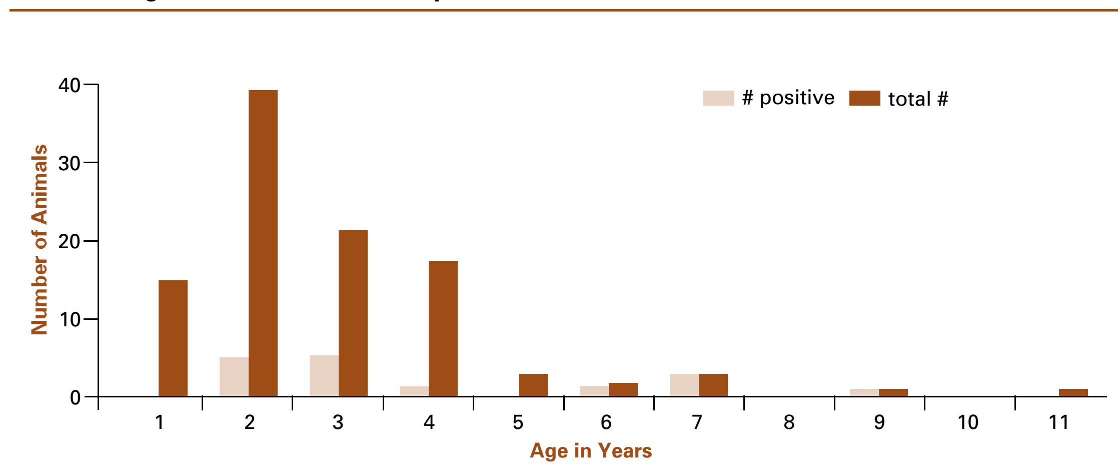


TABLE 2: Accuracy of ELISA testing in a low prevalence herd. True prevalence of *M. avium* subsp. *paratuberculosis* infection = 1%, assumed test sensitivity = 60%, specificity = 97%.

Test result	Infected cows	Non-infected cows	Totals
ELISA positive	6	30	36
ELISA negative	4	960	964
Totals	10	990	1000

Positive predictive value = 6/36 = 16.7%
Negative predictive value = 960/964 = 99.6%

Table 3: High prevalence herd. True prevalence of *M. avium* subsp. *paratuberculosis* infection = 30%, assumed test sensitivity = 60%, specificity = 97%.

Test result	Infected cows	Non-infected cows	Totals
ELISA positive	180	21	201
ELISA negative	120	679	799
Totals	300	700	1000

Positive predictive value = 180/201 = 89.6%
Negative predictive value = 679/799 = 85.0%

infected animals and acquired by young animals through ingestion of contaminated water and feed. In general, it is believed that younger animals are more susceptible, but exact susceptibilities are not known. *M. avium,* subsp. *paratuberculosis* organisms are also found in the milk and colostrum of infected animals.This is a source of infection for calves.Studies have also looked into pasteurization methods of milk for human consumption. Some have suggested that the organism survives pasteurization, while others have failed to confirm this.

Types of tests

The *sensitivity* of a test relates to the proportion of infected animals that have a positive test, while the *specificity* relates to the proportion of noninfected animals that have a negative test.The three tests to focus on for John's disease are fecal culture, ELISA,and AGID. Other tests exist but have limited practicality or clinical use at this point in time. ELISA and AGID are serological tests that detect antibodies produced by the cow in response to infection with *M. avium*, subsp. *paratuberculosis.* AGID is a direct antibody test,while ELISA is an indirect antibody test.

Fecal culture:This test is the gold standard for Johne's disease. It has a sensitivity of 30%-40%, but the specificity is nearly 100%.The high specificity means that few false positives will occur. However, the low sensitivity will result in many false negatives in tested animals that are intermittently shedding organisms,especially early in the disease. The test is expensive to run and the culture time is several months and requires considerable lab space. Positive cultures can be diagnosed by as early as 6 weeks if they show good growth; however, a negative culture takes 16 weeks to confirm.

AGID: The agar gel immunodiffusion (AGID) test is considered to be close to 100% specific. This simple overnight test can be used to confirm the diagnosis in clinical cases.It is not a good screening tool because it is less sensitive than ELISA

***Absorbed ELISA*:** In the enzyme-linked immunosorbant assay (ELISA) test, the word 'absorbed' refers to a step in the procedure where an extract of *Mycobacterium phlei* is added to reduce cross-reactions and false positives.The manufacturers (IDEXX) report the sensitivity to be 60% and the specificity to be 97% in individual animals. Other groups, such as the US National Johne's Working Group, have reported the sensitivity to be about 25%.

The positive and negative predictive values of the test vary dramatically with the prevalence of disease within the herd.An example of a low and high prevalence herd is used to show the accuracy of the ELISA test, depending on the prevalence. The manufacturer reported sensitivity of 60% and specificity of 97% in these examples with a herd size of 1000 animals (Table 2 and Table 3).

The positive predictive value (PPV) in the low prevalence herd is only 16.7%.This means the probability that a positive test indicates a truly positive cow in this situation is only about 17%.This increases to nearly 90% in the high prevalence herd. Conversely, the negative predictive value (NPV) is the probability that a negative test indicates a truly negative animal. In the low prevalence herd, this is nearly 100%, but it decreases to 85% in the high prevalence herd. In the low prevalence herd,the risk of false positives is fairly high (83.3%).In a high prevalence herd,the risk of a false negative (15%) is higher than in the low prevalence herd.Using a sensitivity of 25% as reported by the American National Johne's Working Group, the PPV and NPV at the different herd prevalences vary from these values by only a few percent.

With the ELISA, the results obtained show the amount of antibody within a sample and a cutoff value

TABLE 4: Recommendations for management of Johne's disease in a beef cow calf herd

Discussions with the producer
- State the reasons for getting involved with a Johne's Management Program
- Set realistic goals
- Assess the producer's expectations and commitment to carrying out the recommendations

Observation and history taking
- Obtain a complete history of the disease in the herd.
- Obtain the date, age, and source of the first clinical case
- Obtain the date, age, and source of subsequent cases, including clustering in family groups, age cohorts, or locale of animals
- Look for obvious risk factors, such as clinical cases, calving management, water sources, and recruitment into the herd.
- Eliminate clinical cases immediately
- Blitz ELISA testing of the entire herd (at pregnancy diagnosis time)
- Testing in the fall allows several months to remove test positives before calving
- Confirm cases with fecal culture if owner desires
- When Johne's disease has been definitively diagnosed in a herd, the ELISA test is quite accurate for a herd screen, as false positives are less likely

Eliminate all test positives
- The test positive animals need to be eliminated so they are not brought into the calving pens
- In the event that there are a large number of test positive animals that the producer does not want to eliminate immediately, the positive animals could be managed in separate "dirty" facilities, allowing slower culling
- Retest the herd in 6 months (before turnout to pasture)
- The test positives could be either slaughtered or moved to a dirty pasture (ie, winter wheat that could be later plowed under)
- Test yearly at time of pregnancy diagnosis
- Remove positives by calving time to help prevent contamination of the calving area

is given that indicates a positive animal.The actual value for each sample can also be adjusted through comparison positive and negative controls. This result is reported as a sample to positive ratio (S/P). This provides further information. Looking at a sample to positive ratio and using the lab assessment can identify other positive or suspicious animals. ELISA is a rapid,low cost test with results available in 24 hours. For many laboratory tests, we assume the sensitivity of a test to remain constant. In the case of Johne's disease, the ELISA sensitivity increases in animals with clinical signs.In subclinical cases, the sensitivity is reported as 15% +/- 6.6%.In clinical cases,it is 87% +/- 8.4%.This means fewer false negatives in clinical cases.

Testing summary

• In order to increase the accuracy of testing in animals showing no clinical signs, use two or more different kinds of tests (ie, use fecal culture to confirm a positive ELISA result).The AGID is not a different kind of test than the ELISA because they are both based on antibody serology, so fecal culture is more appropriate as a confirmatory test for ELISA.

• It has been reported that the real prevalence of Johne's disease in a herd is about double the ELISA

Table 5: Management procedures

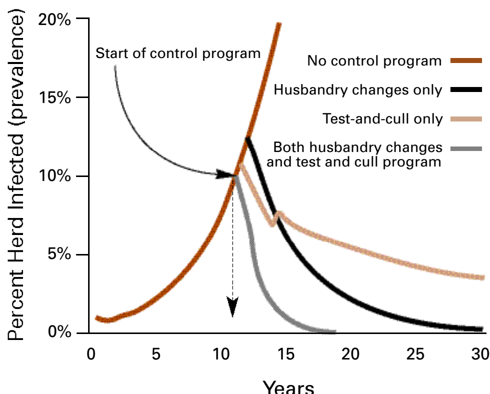
Calving area
- Lateral and vertical transmissions are facilitated by a tight calving season and high stocking densities
- Decrease the possibility of spread to calves by minimizing environomental contamination
- To further reduce probability of disease spread at calving season, spread out animals and keep bedding pack clean
- Every 10-14 days, move cows that have not calved to a clean area so that young susceptible calves are born in as clean an area as possible
- Consider timing of calving season so animals can be more spread out

Watering areas
- Prevent fecal-oral transmission
- Fence off stagnant watering areas
- Use raised waterers

Pasture area
- Harrow areas with contaminated manure
- Consider how manure is moved through the farm and eliminate potential contamination of areas where calves are kept

Cull test positive families

FIGURE 2: A computer simulation of effects of different Johne's control programs. This is based on a 100 cow milking herd where one cow was infected with Johne's disease at year one.



(Courtesy of Dr. Mike Collins, School of Veterinary Medicine, University of Wisconsin.)

prevalence. Others have suggested that the real prevalence is 10 to 25 times the number of clinical cases.

Recommendations for preventing, diagnosing, and managing Johne's disease in a beef cow calf herd are shown in Tables 4 and 5.

Summary

Johne's disease is emerging as a disease of major importance to the cattle industry due to its significant economic impact on many herds.Breed associations are wondering how to certify herds of purebred animals for sale. The public and media are concerned with food safety issues.The possibility of the causative organism of Johne's disease being involved in Crohn's disease is an emerging food safety concern.The veterinary and cow-calf industries need to be pro-active in the eradication of Johne's disease. Further research into the pathogenesis of Crohn's disease is necessary as most of the research concerning this disease has been based on dairy animals and does not apply to the cow-calf industry in western Canada. The recommendations from the United States are based on very different costs of testing and again are difficult to apply to the situation in this country.

It is important to take what we know about this disease and develop an interim strategy for managing it while we wait for further research that is relevant to cow-calf producers in western Canada. Initially, the aim should be for disease reduction. It will take time to make significant changes in prevalence. Combining two or more control strategies will help the disease prevalence to decrease faster more rapidly. Figure 2 shows a computer simulation of the decrease in prevalence over time in Johne's disease with different control programs.

Further research is required to identify critical control points in the spread of Johne's disease. Then we will have a better idea of when most animals become infected and at what rates. We also need to know how long infected pastures remain a risk,and what type of ground water contamination occurs with this disease. Encouragement from the industry to develop herd certification programs for seedstock producers would aid in motivation for producers. Hopefully this disease will not become a recognized zoonosis. However, if it is, action now will reduce our problems in the future.

Further Readings

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Abstract of Interest

Causation of Crohn's Disease by *Mycobacterium avium* subspecies *paratuberculosis*

HERMAN-TAYLOR, J. , BULL, T.J., SHERIDAN, J.M., CHENG, J., STELLAKIS, M.L., SUMAR, N.

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a member of the *M avium* complex (MAC). It differs genetically from other MAC in having 14 to 18 copies of IS900 and a single cassette of DNA involved in the biosynthesis of surface carbohydrate. Unlike other MAC, MAP is a specific cause of chronic inflammation of the intestine in many animal species, including primates. The disease ranges from pluribacillary to paucimicrobial with chronic granulomatous inflammation like leprosy in humans. MAP infection can persist for years without causing clinical disease. The herd prevalence of MAP infection in Western Europe and North America is reported in the range 21% to 54%. These subclinically infected animals shed MAP in their milk and onto pastures. MAP is more robust than tuberculosis, and the risk that is conveyed to human populations in retail milk and in domestic water supplies is high. MAP is harboured in the ileocolonic mucosa of a proportion of normal people and can be detected in a high proportion of full thickness samples of inflamed Crohn's disease gut by improved culture systems and IS900 polymerase chain reaction if the correct methods are used. MAP in Crohn's disease is present in a protease-resistant non-bacillary form, can evade immune recognition and probably causes an immune dysregulation. As with other MAC, MAP is resistant to most standard antituberculous drugs. Treatment of Crohn's disease with combinations of drugs more active against MAC such as rifabutin and clarithromycin can bring about a profound improvement and, in a few cases, apparent disease eradication. New drugs as well as effective MAP vaccines for animals and humans are needed. The problems caused by MAP constitute a public health issue of tragic proportions for which a range of remedial measures are urgently needed.

Can J Gastroenterol 2000 Jun;14(6):521-39

Current Continuing Education

Johne's Disease Articles No. One to Four

HANSEN, D. AND ROSSITER C., AABP FOOD SAFETY COMMITTEE 2000

Edited and reviewed by the National Johne's Working Group, and endorsed for publication by the USAHA. These papers include:

- *Clinical description and epidemiology of Johne's disease in cattle*
- *Critical Management Points for Prevention and Control of Johne's Disease in Dairy Cattle*
- *Critical Management Points for Prevention and Control of Johne's Disease in Beef Cattle*
- *Concepts for Interpretation of Johne's Disease Diagnostic Tests*
- *Johne's Disease Diagnostic-the ELISA*
- *Johne's Disease Diagnostic Tests-Fecal Culture*
- *Testing-Choosing the Right Test for the Right Purpose*

These are current, concise, and informative veterinarian directed articles. The disease and the diagnostic tests are thoroughly discussed in a useable format. These articles are distributed through the AABP.

Popular Websites

www.vetmed.wisc.edu/pbs/johnes
The Johne's Information Center
Dr. Michael T. Collins
School of Veterinary Medicine
University of Wisconsin

www.paratuberculosis.org/
International Association for Paratuberculosis Inc.

www.crohns.org

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