Review Article Compte rendu

Johne's disease in Canada
Part I: Clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds

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Abstract — Recent international developments in the area of infectious disease control and nontariff trade barriers, along with possible zoonotic concerns, have provoked a revival of interest in Johne's disease in Canada and elsewhere. The bacterium causing Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis,* is distributed worldwide and causes chronic granulomatous enteritis, also known as paratuberculosis, in domestic and exotic ruminants, including cattle. The subclinical form of this disease results in progressive weight loss, reduced milk production, lower slaughter value, and premature culling, with possible impacts on fertility and udder health. Eventually, infection can lead to the clinical form that manifests as chronic diarrhea, emaciation, debilitation, and eventual death. Currently, available tests to detect infected animals produce many false-negative results and some false-positives, particularly in subclinically infected animals, thus making their interpretation and utilization challenging in control programs.

The objective of this 2-part review is to critically review the literature about Johne's disease in dairy cattle for bovine practitioners in Canada. Part I covers the clinical stages, pathophysiology, diagnosis, and prevalence of infection in Canada, while Part II discusses impacts, risk factors, and control programs relevant to Canadian dairy farms. By reviewing the scientific literature about Johne's disease, control of the disease could be pursued through informed implementation of rational biosecurity efforts and the strategic use of testing and culling.

Résumé — Maladie de Johne au Canada — Premier volet : Symptômes cliniques, physiopathologie, diagnostic et prévalence dans les troupeaux laitiers. Les récentes avancées internationales au niveau du contrôle des maladies infectieuses, les barrières commerciales non tarifaires et les craintes d'une éventuelle zoonose ont ravivé l'intérêt pour la maladie de Johne au Canada et ailleurs dans le monde. La bactérie responsable de la maladie de Johne, *Mycobactérium avium* sous-espèce paratuberculosis, a une distribution mondiale et cause une entérite granulomateuse chronique, connue également sous le nom de paratuberculose, chez les ruminants domestiques et exotiques, incluant les bovins. La forme subclinique de la maladie entraine une perte progressive de poids, une réduction de la production laitière, une perte de valeur à l'abattage, une réforme prématurée ainsi que des répercussions possibles sur la fertilité et la santé du pis. Éventuellement, l'infection peut évoluer vers la forme clinique, qui se manifeste par une diarrhée chronique, une émaciation, un affaiblissement et éventuellement la mort. Les tests disponibles pour détecter les animaux infectés donnent couramment plusieurs faux résultats négatifs et quelques faux positifs, particulièrement pour les formes subcliniques, rendant leur interprétation et leur utilisation contestable dans les programmes de contrôle.

L'objectif de cette revue à 2 volets est de revoir de façon critique la littérature concernant la maladie de Johne chez les bovins laitiers pour les praticiens du Canada. Le volet 1 couvre les stades cliniques, la physiopathologie, le diagnostic et la prévalence de l'infection au Canada alors que le volet II discute des impacts, des facteurs de risques et des programmes de contrôle applicables aux fermes laitières du Canada. En revoyant la littérature scientifique sur la maladie de Johne, la lutte contre la maladie pourrait être poursuivie par une mise en œuvre avisée de mesures rationnelles de biosécurité et par une utilisation stratégique de tests et de réforme des animaux.

(Traduit par Docteur André Blouin)

Can Vet J 2006;47:874–882

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Introduction

Paratuberculosis, or Johne's disease (JD), is a chronic infectious enteritis of domestic and wild ruminants. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a hardy, slow-growing, gram-positive, and acid-fast bacterium (1,2). Despite having 99% DNA homology (3), MAP can be differentiated phenotypically from *M. avium* subspecies *avium* and *M. avium* subspecies *sylvaticum* by its dependence on mycobactin (4), and genotypically by the presence of multiple copies of an insertion element, IS900 (5,6).

Restriction endonuclease analysis has identified variations in 2 principal types of MAP, a cattle type (C) and a sheep type (S), that were first identified by Collins et al (7). Other variations have also been identified, although their importance is unclear (8–10). Paratuberculosis in cattle, goats, deer, and camelids is caused mainly by type C, whereas sheep are usually infected by type S. However, the cattle type can infect sheep and vice versa (11).

The natural hosts for MAP are wild and domesticated ruminants, including dairy and beef cattle, sheep, goats, red deer, cervids, and camelids (12). However, other nonruminant wildlife, such as the fox, weasel, crow, rat, wood mouse, rabbit, hare, and badger, have also been found to harbor MAP (13). Calves inoculated with MAP from a free living rabbit developed typical histological lesions consistent with Johne's disease, demonstrating that wild animals other than ruminants may also contribute to the spread of the disease (14). However, calves are more likely to be exposed to manure from other mature cattle than from wildlife; therefore, the major sources of infection on most farms are likely to be infected domesticated ruminants that shed the bacterium in their feces. The route of infection is usually through ingestion, be it contaminated water, milk, or feed. The purpose of Part 1 of the 2-part series of review articles is to critically review the literature on clinical stages of JD, pathophysiology, diagnostic and screening tests, and prevalence estimates of infection in Canada to enable bovine practitioners in Canada to successfully implement control strategies, discussed in Part 2.

Methods

Due to substantial differences in management and production between dairy and beef cattle, and the extensive literature on JD for both dairy and beef cattle, this paper focuses on dairy cattle. Also, because the intended audience for this paper is primarily veterinarians in Canada, we have emphasized Canadian references as much as possible.

Medline (accessed via PubMed from 1950 to present), The Commonwealth Animal Bureaux (CAB) (accessed via VetCD and ParasiteCD from 1973 to present), and Agricola, produced by the National Agricultural Library of the U.S. Department of Agriculture (accessed via the National Agricultural Library from 1970 to present) were used to collect the majority of the references that were used in this paper. The keywords used in the search of the databases were Johne's disease or paratuberculosis, Canada or Canadian, dairy and cattle. In addition, a number of papers were included from the reference lists of other papers, or personal knowledge of reports or conference proceedings, where the literature search did not identify papers with salient information for this review.

All relevant material collected from the above process was included in the review, provided that it was pertinent to the methods of production within the Canadian dairy industry. Material was only excluded if information was redundant or outdated and had been directly refuted. Otherwise, all available information was included.

Pathophysiology

Ingested MAP bacteria enter the intestinal wall through the small intestinal mucosa, primarily in the region of the ileum, via M cells (specialized absorptive mucosal cells) residing in the Peyer's patches (15). Where they are resistant to intracellular degradation, they are eventually phagocytosed by subepithelial macrophages (16). While the bacteria are in the mucosal tissue and submucosal macrophages, there is little or no detectable reaction to the infection. This delayed detectable humoral immune response is one reason for the poor sensitivity (Se) of serological diagnostic tests for MAP, as explained in detail later.

Eventually, the infected macrophages migrate into local lymphatics (17,18), spreading the infection to regional lymph nodes. In the regional lymph nodes, the organisms are capable of stimulating inflammatory and immunological responses (19). The immune response towards MAP resembles that of other mycobacterial infections. Most animals mount a cellular immune response involving a variety of cells, most importantly T lymphocytes (20). Cytokines produced by T helper cells also contribute to the protective response against mycobacterial infections, especially the cytokine gamma interferon (IFN-γ). Production of IFN-γ has been recognized as a key step in resistance against mycobacterial diseases in general, and it may provide a means to help monitor early infection in some animals (21). In some cows, the cellular immune response has been shown to be able to control the infection, with the cows never developing clinical signs but remaining subclinically infected for life (22). In those animals in which the cellular immunity is unable to control the disease, a detectable humoral immune response will develop, along with increased shedding of bacteria (22).

Typically, the organism proliferates slowly in the ileal mucosa and regional lymph nodes. However, poor nutrition, stress related to transport, lactation, parturition, and immunosuppression by agents like bovine viral diarrhea virus have been proposed as accelerating or precipitating the onset of the clinical phase of infection (23).

The physiological mechanism for development of diarrhea in clinically affected animals is thought to be related to antigen-antibody reactions in infected tissue, with subsequent release of histamine (24–26). Macroscopic lesions, if present, are seen primarily in the intestine and it's draining mesenteric lymph nodes, more specifically in the region of the ileum, although they can occur throughout the whole length of the intestinal tract. The intestinal wall is thickened and edematous, and the mucosa has exaggerated transverse folds, mimicking the appearance of corrugated cardboard. The serosal and mesenteric lymphatic vessels are dilated and thickened. Subsequent muscle atrophy, emaciation, alopecia, renal infarcts, anemia, and

leukopenia are thought to be mediated by cytokines (23,27). There is no evidence to suggest that the pathophysiology or progression of disease differ between infected cattle in Canada and elsewhere.

Clinical effects and stages of paratuberculosis

Infection with MAP can be divided into 4 stages as described by Whitlock and Buergelt (28), depending on the severity of clinical signs, potential for shedding organisms into the environment, and the ease with which the disease may be detected by using current laboratory methods.

Silent infection

This stage generally includes young stock up to 2 y of age; it is called "silent" because 1) there are no clinical signs of infection, 2) there are no measurable subclinical effects of infection, and 3) there are no cost-effective diagnostic tests that can detect infection. The only means of detecting infected cattle at this early stage is by demonstration of the established organism in the intestinal tract, either by culture or by histologic demonstration of microgranulomas in the intestine or regional lymph nodes, a cost-prohibitive procedure if multiple animals require testing. Other diagnostic tests, such as johnin (sterile solution of growth products of Johne's bacillus) skin testing and gamma-interferon tests that utilize the cell mediated response (CMI), have also been used to detect this stage of the disease. However, there are common antigens between MAP and other environmental *Mycobacterium* spp., resulting in low specificity (Sp) for these tests (29,30), making them ineffective as routine screening tests. Infected animals in this stage may shed infectious organisms into the farm environment at levels below the threshold of detection (31).

Subclinical infection

Animals with subclinical MAP infection do not yet have clinical signs of infection, but may be detected as infected by using cost-effective diagnostic tests and may begin to have measurable effects of infection (as discussed in Part 2 of the series) (32,33). Some of these infected cattle may be detected by fecal culture and subsequently removed from the herd. However, focal lesions, variable rates of disease progression and shedding, and dilution of organisms in large volumes of intestinal content result in intermittent detection of fecal shedding (34). Therefore, other infected animals test negative by using current fecal culture techniques, yet they may be shedding low numbers of organisms in the manure, which contaminate the environment and pose a threat to other animals on the farm. Some animals may have detectable antibodies to MAP, an altered cellular immune response, or both, particularly if they are getting close to entering the next stage of the disease (clinical phase) (12). However, MAP fecal shedding usually occurs before a detectable antibody response (35).

Clinical infection

Initial clinical signs follow a prolonged incubation period of 2 to 10 y, depending on the exposure level and the capacity of an animal to fight the infection (36,37). The first apparent sign is gradual weight loss, despite a normal or, occasionally, an increased appetite. During a period of 3 to 6 mo, concurrent with the weight loss, the manure consistency becomes more fluid. The diarrhea may be persistent or intermittent, at first, with periods of normal manure consistency. Thirst is usually increased and milk production is decreased. However, appetite and vital signs (heart rate, respiratory rate, and temperature) remain normal (28).

Most animals at this stage have a positive fecal culture and have increased serum antibody levels detectable by the commercial enzyme-linked immunosorbant assay (ELISA) and agar gel immunodiffusion (AGID) test. It is estimated that only 10% to 15% of infected animals survive to this stage of infection, because they are often culled due to reduced productivity earlier in the subclinical stage (38). One study showed that out of 90 herds that did not report having a clinical case of JD in the previous 5 y, only 35 herds were completely test-negative during 9 pooled fecal-culture tests done every 6 mo (39).

Advanced clinical infection

Clinically affected animals, if not culled, become increasingly lethargic, weak, and emaciated. "Water-hose" or "pipestream" diarrhea, hypoproteinemia, and intermandibular edema (bottle jaw) characterize the advanced stage of the disease. In the last stage of JD, cows become cachectic, anemic, and too weak to rise (40). Most animals are culled from the herd before this time due to the chronic or intermittent diarrhea, decreased milk production, and or weight loss in the earlier stages of disease (28).

Diagnosis

The diagnostic tests to detect infection with MAP can be categorized into those that identify the organism and those that identify an immunological reaction to the organism. Evaluation of the performance of diagnostic tests is typically done by comparing estimates of Se (ability of the test to detect infected cattle) and Sp (ability of the test to identify healthy cattle), based on a "gold standard" that has identified animals as truly infected and truly noninfected. However, comparisons of the Se and Sp of diagnostic tests for MAP should be interpreted with great caution, because there are a number of factors that have a major impact on these estimates, including 1) the type of gold standard used, 2) the stage of infection of the study animals, and 3) the type of farms utilized to source animals for testing. Each of these will be discussed in turn.

Due to delays of 2 to 10 y between time of infection and development of measurable immune system reactions and shedding, various "gold standards" have been utilized for MAP-infection status in the past. Tissue culture of MAP is considered the ideal gold standard test, because, even before fecal shedding or an immune response is present, it can detect growth of MAP in multiple organs, including the intestinal mucosa and submucosa, and regional lymph nodes (41). However, fecal culture has been used as the gold standard in many studies (42–48) in the past, due to the high cost and logistical difficulties of sampling for tissue culture. The methods for fecal and tissue culture are identical and are described below.

Table 1. Characteristics of currently available diagnostic tests for Johne's disease in Canada

		Sensitivity			
		Subclinical cow			
		Low prevalence herds and/or low shedders[a]	High prevalence herds and/or high shedders[b]	Clinical cow	Specificity
Fecal culture	Estimate	19%[c]	53%[d]	> 90%[e]	Approaching 100%[e]
	Reference	(56)	(56)	(49)	(49)
Serum ELISA[n]	Estimate	12%[f]–15%[f]	40%[g]–75%[g]–95%[h]	87%[i]–88%[i]	96.8%[j]–99%[i]
	Reference	(57)–(42,43)	(57)–(42,43)–(58)	(43)–(42)	(42)–(56)
Cow milk ELISA[n]	Estimate	—[k]	51%[l]–84%[h]	—[k]	92%[i]–96%[i]
	Reference		(58)–(58)		(59)–(59)

[a]Low prevalence herds (< 25%) and/or low shedders (< 10CFU[m])
[b]High prevalence herds (≥ 25%) and/or high shedders (≥ 10CFU)
[c]Tissue culture was gold standard in low prevalence herds
[d]Tissue culture was gold standard in high prevalence herds
[e]Tissue culture was gold standard
[f]Fecal culture was gold standard in low shedders
[g]Fecal culture was gold standard in high shedders
[h]Fecal culture was gold standard in high shedders in high prevalence herds
[i]Fecal culture was gold standard
[j]Tissue culture or fecal culture or history of herd was gold standard
[k]No published reports found
[l]Fecal culture was gold standard in low shedders in high prevalence herds
[m]Colony forming unit
[n]Enzyme linked immunosorbant assays

The delays in immune response or shedding also mean that when tests are utilized on animals with clinical JD, they will have a better Se than when they are used on animals with subclinical JD (43,49), because the clinically affected animals are much more likely to be shedding bacteria or have developed a detectable immune response (19). False-negative test results are common, particularly in calves, heifers, and even 1st lactation cows (cattle in silent and subclinical stages) (50). Test results from animals with subclinical paratuberculosis can be a challenge to interpret, because clinical signs are not present to assist in their interpretation (42,49,51).

Regarding farm type, on farms with a known history of clinical JD, there will likely be more bacteria in the environment and higher exposure of youngstock to MAP than on farms without a history of clinical JD. If youngstock undergo higher exposure to MAP on a particular farm, they are more likely to develop a detectable immunological reaction or to begin shedding bacteria earlier in life (24,25,52), leading to higher detectable prevalence of infection on that farm and increased shedding of bacteria in those infected cattle on those farms compared with farms where there is lower exposure to MAP. Therefore, evaluation of test performance in cattle on these farms will lead to higher estimates of Se and Sp than on farms without history of clinical JD.

As a result, in Table 1, the test Se of the most widely used diagnostic tests for MAP infection are categorized with respect to testing for clinical versus subclinical infection, and to testing for subclinical infections in farms with high prevalence (≥ 25%) of MAP infection versus in those with low prevalence (< 25%).

Identification methods

1. Culture on tissue or feces (individual and pooled samples)

A number of different media have been used to culture MAP. The standard culture procedure utilized in Canada is Herrold's egg yolk medium (HEYM); however, culture time is often 16 wk before observable growth is seen for this slow-growing bacterium (28). A radiometric system has been developed that reduces the culture time by half, because detection of growth is not visual but through the detection of metabolized radioisotopes in the media (53). However, because the system requires expensive safety equipment to handle the radioisotopes, at the time of publication, only laboratories in British Columbia (Animal Health Monitoring Laboratory, Abbotsford) and Ontario (Animal Health Laboratory, Guelph) are offering this system on a commercial basis, while the Manitoba provincial laboratory (Veterinary Services Branch, Winnipeg) is conducting research and development with the system. Recently, a specialized broth media system has been developed that has reduced the detection time to 6 wk, without loss in test Se, through the detection of alterations in oxygen, CO_2, or pressure within a sealed bottle (54). At the time of publication, the only Canadian laboratory currently offering broth culture testing is the Atlantic Veterinary College in Charlottetown, Prince Edward Island (PEI). The Agri-Food Laboratories Branch laboratory in Edmonton, Alberta, does MAP cultures, using the standard HEYM method, but only for research purposes and for some of the samples submitted under the Alberta Johne's Control Program.

If bacterial growth is detected, the bacterium is isolated and its identity is confirmed through the morphologic characteristics and mycobactin dependency of the bacterial colonies, acid-fast staining, and, sometimes, the detection of the insertion sequence IS900 by polymerase chain reaction (PCR). With identification of MAP, the animal is considered infected. However, laboratory error can occasionally lead to cross-contamination and false-positive test results (55). Also, the phenomenon of "pass-through" of bacteria through the gastrointestinal tract could lead to other false-positives, but this remains a hypothesis and has not been fully substantiated. Therefore, Sp of fecal culture is described as being virtually 100% (49) and fecal culture

Table 2. Seroprevalence estimates of bovine paratuberculosis at the animal and herd level in Canadian dairy herds (from East to West)

Province (Reference #)	Number of herds	Number of animals	Animals test +ve[a] (%)	Herds with 1 test +ve[b] (%)	Herds with 2 test +ve[c] (%)	Animals test + in herds with 1 test +ve[d] (%)	Animals test + in herds with 2 test +ve[e] (%)
Nova Scotia (77)	30	814	3.3	53.3	16.7	5.3	8.1
Prince Edward Island (77)	30	816	1.3	33.3	16.7	5.0	6.3
New Brunswick (77)	30	804	2.9	43.3	16.7	6.4	11.0
Ontario (78)	51	1530	2.2	37.0	9.8	4.6	8.4
Manitoba (79)	40	1204	4.5	68.4	43.1	6.6	8.7
Saskatchewan (80)	51	1530	2.7	43.6	24.2	6.3	8.6
Alberta (81)	50	1500	7.0	74.0	40.0	—[f]	—[f]

[a]Animals testing positive
[b]Herds with at least 1 animal testing positive
[c]Herds with at least 2 animals testing positive
[d]Animals testing positive in herds with at least 1 animal testing positive
[e]Animals testing positive in herds with at least 2 animals testing positive
[f]Published report did not include these figures

is considered an excellent confirmatory diagnostic test of paratuberculosis for animals that test positive to immunological tests (Table 1).

Due to the lengthy duration of testing and the specialized equipment and media required for culturing MAP, the cost per sample tested is high ($35 to $60/sample). Therefore, pooling fecal samples has been utilized to test large numbers of animals for less cost per animal (60), while still maintaining reasonable Se to detect infected animals (61). In comparing conventional culture to pooled culture, 94% of pooled samples with cows with moderate to high numbers of MAP yielded positive culture results (62). Pooled fecal culturing has been shown to have a herd level Se of 73% (60), meaning that 73% of infected herds were detected with a single set of strategically (by age cohorts) pooled samples. However, the maximum number of negative animals that can be mixed in with a sample from a positive animal (while still getting a positive pooled test) needs to be determined, and this number will need to be appropriate to infection prevalence, severities, and shedding levels seen in Canada. Based on initial results from outside Canada, 3 to 5 fecal samples in a pool may be the optimal number (60,62,63). Any Canadian laboratory that is equipped to conduct MAP cultures should also be able to conduct pooled fecal cultures.

2. Polymerase chain reaction on feces

Since the discovery of the IS900 insertion sequence, attempts have been made to perform PCR techniques directly on clinical samples (64,65). Through amplification of this piece of genetic material, the PCR is able to provide a much faster result compared with culture techniques, with a turnaround time of 4 d for most laboratories (55). However, PCR is less sensitive than culture due to the presence of inhibitory substances in fecal specimens (66). Another concern is that IS900 may not be as specific for MAP as once believed. The IS900 element has been detected in other mycobacterial strains isolated from the feces of ruminants (67). At the time of publication, PCR testing is being offered commercially in Canada only at BIOVET Inc. in St. Hyacinthe, Québec, and at the Faculté de Médecine Vétérinaire in Montréal, Québec. It is being used as a confirmation of positive cultures where culturing is being done. If PCR were to be done on a large scale, laboratory error could lead to false-positive test results unless very strict adherence to preventing even minute cross-contamination was implemented.

Immunological methods

1. Enzyme linked immunosorbant assays on serum and milk

The main type of immunological test that is widely available and commonly used is the enzyme linked immunosorbant assay (ELISA), which detects an optical density in serum (68) or milk (69,70) that correlates with an antibody response to MAP. The ELISAs have been desirable tests to use because of their ease of sample collection (blood or milk), rapid test results (within a week), and relatively low cost (approximately $10 per sample). However, for several reasons, results from ELISAs need to be interpreted with caution. Due to the long delay between infection and presentation of bacteria to the immune system in sufficient numbers to develop a detectable immune response, the reported Se of the serum ELISAs for detecting subclinically infected cattle are much lower than the Se of fecal cultures, leading to many false-negative results (42,45,46,69). In fact, many studies have estimated the Se of the serum ELISA to range from 15% to 75%, on the basis of the proportion of fecal shedders that were seropositive (42,43,49).

Milk ELISA testing has recently been introduced as another immunological test for detecting subclinically infected cattle, with the obvious practical advantage of ease of sample collection. However, independent, peer-reviewed evaluation of the operating characteristics of the ELISA of milk is still limited. A recent study in Ontario (58) reported only moderate agreement between serum and milk ELISAs, and the milk ELISA detected 12% fewer infected cows than did the serum ELISA. These findings make biological sense considering that antibody concentrations in milk depend not only on levels in serum, but also on milk production (58), parity, and days in milk (71). The added variability in antibody levels in milk relative to serum (72) makes interpretation of results from milk ELISA even more challenging than those from serum ELISA, which have inherent laboratory variability (73). Further research may identify a role for the milk ELISA as a practical method of monitoring MAP infection at the herd level or instigating interest in controlling JD.

Another caution regarding the use of ELISAs in low prevalence herds is that false-positive test results can also be a problem when a large number of cattle are tested with a test that has a Sp that is not very close to 100% (42). While some studies have reported Sp estimates of 99% (43,49,74), another study (42) likely provides a more realistic estimate of Sp (96.8%), because it utilized multiple sources of samples (more representative of the North American dairy cattle industry as a whole), providing more possibility of cross reactivity with other microorganisms to give false-positive test results. Therefore, if an ELISA was used on 100 uninfected cattle, it would likely produce 1 (Sp of 99%) to 3 (Sp of 97%) false-positive test results, which could erroneously categorize an uninfected herd as infected, if confirmatory tests were not utilized on the ELISA-positive cattle. In a low prevalence herd of 100 cows with 5 truly infected animals, the low sensitivity of ELISAs would lead to only 1 of the 5 infected animals likely testing seropositive and 1 to 3 false-positive test results. Therefore, with only 1 of 2 to 4 test positives being truly infected, the predictive values of a positive test result would vary between 25% and 50%, making it difficult to know how to interpret and act on positive test results. Therefore, for apparently healthy cows that are ELISA-positive, the feces should be cultured to confirm infection status, particularly in herds suspected of having a low prevalence of infection. If the fecal culture is negative, these ELISA-positive cows should be retested in 6 to 12 mo, because the owner does not know if these nonshedding ELISA-positive cows are truly uninfected or just not shedding in detectable numbers at the time of sampling.

One additional caution regarding the interpretation of ELISA results relates to the form in which they are reported. Interpretation of results has generally been made on a single cut-off value that allows for dichotomous test results, positive or negative. Although this would appear to make results easier to interpret and allow for Se and Sp calculations, valuable information is lost, because the likelihood of true infection is much higher in cattle with a high optical density, particularly in herds that have a history of clinical JD infection (57). As a result, some laboratories are appropriately utilizing a 3-level result system–negative, suspect, and positive (75), or 4-level result system — negative, suspect, weak positive, and strong positive, based on categorizations of likelihood ratios generated from the optical densities (55).

At the time of publication, there are only 2 serum ELISAs currently offered on a commercial basis in Canada, with different provinces using different ELISAs. It is unlikely that there is a large and significant difference in the test performance of the 2 ELISAs; both tests have difficulty detecting, subclinically infected cattle.

2. Agar gel immunodiffusion

There is one other immunological test that is available in Canada, the agar gel immunodiffusion (AGID) test. It was developed as a quick test for animals that were showing clinical signs of JD. Some reports estimate that when AGID results are positive, there is a 95% chance of actual MAP infection in a clinically affected cow (28). However, the Se of the AGID for subclinical cows is poor, with 1 report of an Se of 18.9% (76). Therefore, use of the AGID is restricted to animals showing clinical signs of JD. The ELISAs are equally sensitive at detecting MAP in clinically affected cattle; and therefore, it is unlikely that the AGID offers any advantage over the ELISA.

Prevalence

The results of seroprevalence studies done recently in dairy cattle in Canada are shown in table 2. Seroprevalence at the animal level in dairy cattle ranged from 1.3% (PEI) (77) to 7.0% (Alberta) (81). At the herd level, 9.8% (Ontario) to 40.0% (Alberta) (81) of herds had at least 2 seropositive cows. The provincial differences in seroprevalence may represent real differences in the distribution of the organism due to variations in management or other risk factors for transmission. Conversely, these seroprevalence differences may be due to variations in sampling and testing protocols. Normally, estimated true prevalences of infection can be calculated to adjust for differences in testing protocols; however, for JD, there is little consensus on the adjustments for test Se and Sp required to calculate true prevalence estimates. Therefore, even comparisons of estimated true prevalences should be interpreted with caution.

All of the provincial studies in Table 2 were conducted by using the same number of animals per herd and the same ELISA (IDEXX ELISA; IDEXX Laboratories, Westbrook, Maine, USA) test (except for Alberta), with the same cut-off value for interpretation of test-positives. However, comparisons between these seroprevalence estimates should be interpreted with caution for a number of reasons. Sera from Manitoba were tested at a separate laboratory from the other provinces, possibly impacting on the survey results. Even the sera that were tested at the same laboratory were tested at different times with different lots of test kits, also possibly impacting on survey results. Furthermore, the Ontario study consisted of herds that were purposely chosen to monitor disease (mastitis) incidence, and likely an underestimate of the true prevalence of paratuberculosis in dairy herds in Ontario. A 1986 to 1989 survey of 14 932 cows in 304 dairy herds in Ontario, using a lipoarabinomannan antigen enzyme-linked immunosorbent assay (LAM-ELISA), found 15.2% of the animals tested seropositive (82). The difference between

Table 3. Seroprevalence estimates of bovine paratuberculosis in dairy herds from major dairy producing countries and states outside Canada (from high prevalence to low prevalence at the animal level)

Country (Reference #)	Areas	Number of herds	Number of animals	Animals test +ve[a] (%)	Herds with 1 test +ve[b] (%)	Herds with 2 test +ve[c] (%)	Animals test + in herds with 1 test +ve[d] (%)	Animals test + in herds with 2 test +ve[e] (%)
USA (83)	Florida	452	4491	17.1	—	—	—	—
USA (84)	Wisconsin	158	4990	7.3	50	—	20	
USA (85)	Michigan	121	3886	6.9	66	44	8	12
USA (86)	20 states	967	31 745	2.5	41	17		
Netherlands (87)	National	378	15 822	2.5	54	28	—	—
Austria (88)	National	2757	11 028	1.9	7	—	—	—
Sweden (12)	National	—	4000	1.2	—	—	—	—
Belgium (89)	National	556	13 317	0.8	18	—	3	—

[a]Animals testing positive
[b]Herds with at least 1 animal testing positive
[c]Herds with at least 2 animals testing positive
[d]Animals testing positive in herds with at least 1 animal testing positive
[e]Animals testing positive in herds with at least 2 animals testing positive

these 2 estimates is unlikely to be due to differences in test performance, because of the similarities in the two tests used (IDEXX-ELISA and LAM-ELISA). A control program for JD did not exist in Ontario during the time between the 2 studies, so that the prevalence of MAP infection is unlikely to have declined between 1989 and 1998.

While it is possible to compare seroprevalences between provinces with the above studies, they are likely underestimates of true prevalences of infection at the animal level, due to the low sensitivities of ELISAs for MAP. Very few studies in Canada have been conducted to determine the prevalence MAP infection in dairy cattle based on fecal culture. In the study in Alberta (81), fecal samples were collected from cows in 50 dairy herds and cultured in pools of 3 samples: 3.4% of pools were found to be culture-positive, meaning that from 3.4% to 10.2% of cattle were test-positive (individual cattle results were not reported), leading to the conclusion that the estimated true herd-level prevalence ranged from 28% to 57%, depending on whether 1, 2, or all 3 individual fecal samples in the positive fecal pools were culture-positive.

While fecal culture testing has a better Se than ELISAs for MAP, it still produces many false-negatives, particularly in young infected cattle that have not yet started to shed the MAP bacterium. A recent prevalence estimate, based on tissue culture testing of ileocecal lymph nodes and ileum from dairy cows at a slaughterhouse in New Brunswick, found that 16.1% of dairy cows were culture-positive for MAP (41). This prevalence estimate is likely to be a close approximation of the true infection prevalence, because culturing the ileum and ileocecal lymph node of the selected animals is better than fecal culturing at detecting cows that are infected but not yet shedding bacteria in their feces.

Many other prevalence surveys have been carried out around the world. A brief summary of recent international seroprevalence estimates from representative samples is provided in Table 3. While comparisons between countries should be conducted with caution, there does appear to be a large variation in the reported seroprevalences between countries and even within countries. Seroprevalences at the animal level in Canada are similar to those in other countries, ranging from 0.8% in Belgium (89) to 17.1% in the USA (Florida) (83). At the herd level, the proportion of herds with 2 or more seropositive cows in Canada (77) was also similar to that in other studies, ranging from 17% for the 20 tested states in the USA (90) to 44% in Michigan, USA (85). Some Scandinavian countries have very low seroprevalence for MAP, leading those countries to seriously consider eradication efforts (91,92).

The significant advances in the quality of the diagnostic tests used to detect MAP make it difficult to determine if the prevalence of MAP infection is increasing. There are very few, if any, data from random samples of the same area over time, using similar diagnostic tests. Before the evolution of ELISAs in the late 80s, most of the initial reports of JD prevalence were limited to slaughterhouse data. Perhaps future studies will address this paucity of data and clarify whether MAP is becoming more prevalent or not.

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