Johne's Disease in Alberta

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■ Take Home Message

‣ Johne's Disease (JD) is a chronic, production-limiting disease that is very hard to detect, infects mostly calves under 6 months of age and stays for life with only a few of the calves showing clinical signs as adults.

‣ Alberta Agriculture and Food (AF) performed several surveys in the last few years to determine the extent of JD in the province.

‣ In beef cattle, JD appears to have a low individual prevalence, but a large geographical distribution in a high number of herds.

‣ In dairy cattle, the prevalence of JD at both the individual animal and the herd level is high with a wide geographical distribution in the province.

‣ Alberta Agriculture and Food coordinates and implements several programs and projects in conjunction with producers and veterinarians to prevent, control and eradicate JD from the herds in the province.

‣ The implementation of best management practices, biosecurity and strategic testing are important tools to reduce the risk of infection to newborn calves and to evaluate the status and progression of any on-farm program.

■ Overview

Johne's Disease (paratuberculosis) is a chronic intestinal infection of ruminants that causes inflammation and fibrosis of the intestinal lining. This results in reduced absorption of nutrients, diarrhea, progressive loss of body condition that compromises overall health and immunity, and eventual death (Radostits et al., 2000). The causative agent is the *Mycobacterium avium subspecie paratuberculosis* (MAP), which is an acid-fast, slow growing gram-positive bacteria (Barrow and Felttham, 1993). The epidemiology of the disease is different in dairy and beef herds mainly due to management factors (Humphry et al., 2006). This results in differences in cow and herd prevalence

between dairy and beef herds. Also the fact that MAP grows very slowly delays the immunological response and detection of the bacterium in serum and in feces. This causes a reduction in sensitivity of the diagnostic tests and high variation in the serology versus fecal culture tests, mainly during early stages of the disease when it remains sub-clinical.

■ Prevalence of Johne's Disease in Alberta

Beef Cattle

The Food Safety Division of the Alberta Agriculture and Food Ministry conducted two baseline surveillance studies to evaluate the prevalence and distribution of JD in the province.

1. In 1998, a preliminary survey was conducted to evaluate the prevalence of JD in beef herds; 226 cow-calf herds in the province were sampled (Ollis and Vanderkop, 2000). In 62 of 72 municipalities, practitioners obtained fecal samples from beef cows during pregnancy checking. Samples were cultured using a method incorporating PCR confirmation that was developed by the Agri-Food Laboratories Branch, and has been accredited by USDA for Johne's disease certification. There were 678 samples suitable for culture from the 226 herds and ten herds were designated positive based on a single positive culture. Eleven (11) of the 678 individual animals were positive, giving an estimated prevalence of JD of 1.6% of beef cows in the province. At the herd level, this provides an estimate that 4.4% of the Alberta beef herds may be positive for JD (Table 1). This initial survey indicated that JD represented a concern for beef herds, however, the lower prevalence indicated that the potential to establish free-herds was feasible. As a result, a herd certification testing-based program was proposed and it was implemented in 2001 under the name of the Alberta Voluntary Johne's Cattle Herd Status Program. The philosophy of this program is that repeated testing is a good method to establish negative herds and to maintain their status. Once the levels of infection are low or when herds are testing free, it is recommended that producers only purchase animals from disease free herds. The economic impact of the disease in beef cattle in terms of reduced feed efficiency, reduced milk production, reduced viability of offspring, decreased productive lifespan, reduced reproductive performance and lower slaughter value has not been estimated.

2.   The results of the previous study were the base to initiate a larger descriptive study in the province (Wu and Ollis, 2001). The aims of this study were to establish a baseline seroprevalence rate and to map the distribution of JD in mature beef cattle of Alberta. The Food Safety Division of Alberta Agriculture with the participation of over two dozens beef practitioners collected a total of 3632 serum samples from 346 producers in 30

Table 1. Studies indicating individual animal and herd prevalence of *Mycobaterium avium* subspecies *paratuberculosis* among dairy and beef herds in Alberta

Type	Authors	Study period	SEROPREVALENCE				MAP IN FECAL CULTURE			
			Individual-level (%)		Herd-level (%)		Individual-level (%)		Herd-level (%)	
			Apparent	TRUE	1 sero+	2 sero+	Apparent	TRUE	1 culture+	2 culture+
Beef cattle	Ollis G et al	1998								
	Wu and Ollis	1999	0.96	2.13			1.6		4.4[a]	
	Scott et al	2002-2003	1.5	1.2	28.5	7.9			14.1	
Dairy cattle	Sorensen O	1999	7	8.1		40.0[b]*		2.7 to 8.1[c]		27.6 to 57.1[c]
	Scott et al	2002-2003	9.1	17.5	70.2	58.8[b]				

[a] Positive culture confirmed by PCR
[b] Apparent prevalence
[c] True prevalence (range based on 1 infected cow/+ fecal pool to 3 infected cows/+ fecal pool)
* True prevalence: 26.8%

*Apparent prevalence results from the number of serological reactors on a sampled population, while the adjusted or true prevalence results from mathematically adjusting this value to the sensitivity and specificity of the test.

counties/municipalities throughout the province with a minimum population size of 2000 head of cattle. This represented over 40% of the total number of geographical locations. The samples were selected randomly from animals two years or older during the period from October to December 1999.

Serum ELISA results revealed that at the herd level, 35 producers out of 346 had detectable antibodies to MAP, with an adjusted prevalence of 2.13% (Table 1). Also, the serological data indicated that MAP is widely distributed in Alberta, since twenty of the thirty (66.67%) counties/municipal districts had at least one positive serological reactor (Figure 1). The study concluded that the prevalence of Johne's disease in beef herds in Alberta remained low, but that MAP is widely distributed in the province. However, the results are a conservative estimate of the prevalence of Johne's in Alberta, due to the low sensitivity of the test, sampling bias and the clinical and immunological nature of the disease.

3. On October 2004, Morgan Scott (official report: Scott, 2004) on behalf of AF, presented a report on seroprevalence and risk factors of Johne's disease in dairy and beef herds. The primary objective of this study was to estimate the individual and herd level seroprevalence of *Mycobacterium avium subsp tuberculosis* and other infectious agents in Alberta beef cow-calf and dairy herds. Another important objective of this study was to examine environmental and agro-ecological factors and to identify on-farm management factors associated with the prevalence of MAP and other infectious agents in cattle of Alberta. A third objective was to assess individual and herd level sensitivity of strategic fecal pool samples (5 and 10) to detect known positive individuals and herds. The results of this study provided important information for the Johne's disease status program to accredited veterinarians working in the region and to animal health authorities.
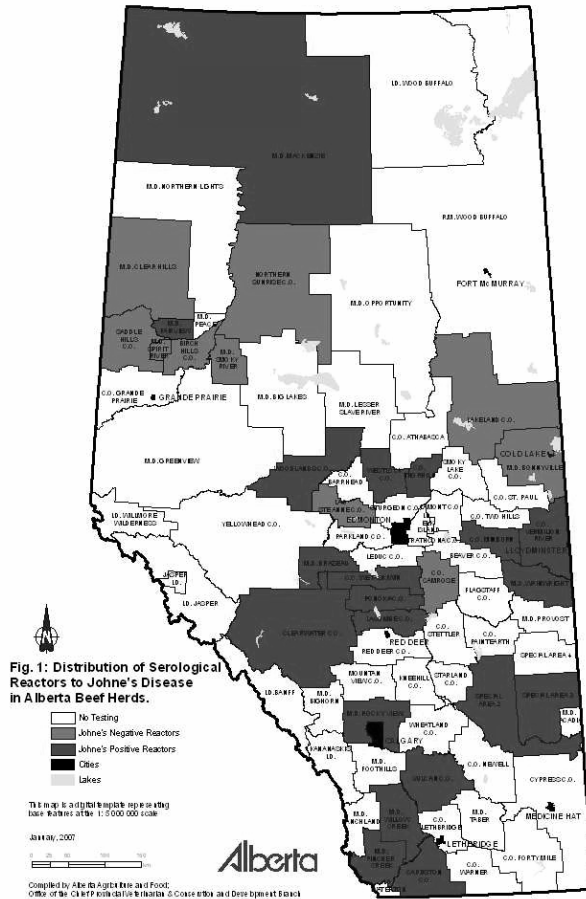
Fig. 1: Distribution of Serological Reactors to Johne's Disease in Alberta Beef Herds.

- No Testing
- Johne's Negative Reactors
- Johne's Positive Reactors
- Cities
- Lakes

This map is a digital template representing base features at the 1:5 000 000 scale

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Figure 1. Distribution of serological reactors to Johne's disease in Alberta Beef Herds. MAP reactors were detected (darker color) in twenty of the thirty (66.67%) counties/municipal districts. Areas in light gray color were negative to MAP. The areas in blank were not tested (From: A Survey to Estimate the Seroprevalence of Johne's Disease in Alberta Beef Herds, Alberta AF, project report, November, 2001).

The study ran from February 2002 through January 2003 and a total of 80 dairy and 110 beef herds were selected from a pool of 288 and 2,989 eligible dairy and beef herds respectively. This study had a wider geographical distribution than the previous one. Beef herd sampling only included 30 adult cattle (over 3 years) per herd. Veterinarians accredited under the Alberta Johne's Control Program were contacted to perform the sampling for both

dairy and beef herds. Blood was collected from the tail vein while individual fecal samples were collected from the rectum using individual disposable gloves. Herd identity and research communications were protected and data was entered into a secured network database. Initially only a positive serum ELISA was confirmed by fecal culture. However, if a fecal culture was positive, then all fecal samples in a farm were cultured, as well as strategically pooled sets of five and ten.

The Scott study reported that the animal-level seroprevalence of MAP infection in adult beef cattle in Alberta was 1.5%. The estimated true prevalence of infection was 1.2%. After combining this result with the results of the confirmatory fecal culture, it was found that 0.6% of adult beef cattle were estimated to be infected with MAP. At the herd-level, it was found that 28.5% of beef cow-calf herds had at least one animal sero-positive for MAP. When the cut point of two seropositives per farm was used to confirm a farm as MAP-positive, the prevalence was 7.9%. By fecal culture, 14.1% cow-calf herds had at least one confirmed fecal culture MAP positive animal (Table 1). In herds known to harbor at least one culture positive animal, the pooling sensitivity to detect MAP, as compared to individual fecal samples, was 73% and 77% for pools of 5 and 10 respectively. On the other hand, fecal pools of five samples correctly identified herds as positive 92% of the time. At the herd level, ecological region, aridity and soil pH were important determinants influencing the risk of MAP presentation.

The study concluded that at the animal-level MAP seroprevalence in beef cattle in Alberta was rare by itself (1.5%) and was very similar to that in a previous study and also to what is seen in many other parts of the world. The efficiency of the diagnostics tests remained low and is one of the limiting factors to successfully identify and control this disease. However, the performance of the fecal test was more consistent in beef compared to dairy cattle, which favors its implementation in a test status program. The use of fecal-pooled samples had good sensitivity at the herd level, but had lower sensitivity at the individual level. The difficulty in identifying animals with subclinical disease is a critical issue that has a negative impact on control and eradication.

In summary, at the animal level JD exhibits a low prevalence in the beef cattle population in Alberta. However, at the herd level and in terms of geographic distribution, MAP has a larger prevalence and a wide distribution among the province. However, when the herd level prevalence based on fecal culture is compared to the results of the 1998 survey, it shows that the number of herds with at least one positive animal has increased almost 3 fold. This is a matter of concern for the beef industry since JD may represent a threat to production efficiency and market access.

Dairy Cattle

1.   During the spring of 1999 a survey to estimate the prevalence of *Salmonella spp* and JD in Alberta dairy herds was initiated by the Food Safety Division of Alberta Agriculture (Sorensen et al. 2003). Two hundred dairy farms that were registered with the Dairy Herd Improvement (DHI) program were contacted by mail and invited to participate. Initially 53 farms agreed to participate, but only 50 herds (most of them located in central Alberta) could be fully sampled. The only criterion for a farm to participate was that it should have at least 45 cows being milked at the time of the initial contact. Out of these cows, a convenient selection was performed of 44 to 45 cows per herd and blood and feces were individually collected. All serum samples were analyzed by ELISA but only 30 samples/herd were randomly selected for statistical analysis and a herd was considered positive if two or more animals reacted to the test. Fecal samples were pooled in groups of three cows and cultured for the presence of MAP.

The results of this study indicated that by serum ELISA, 105 (7.0%) out of 1500 serum samples had antibodies against MAP, giving an estimated true animal-level prevalence of 8.1%. The number of infected herds was 20 (40%), based on two or more individual serum positives in the herd. The true herd-level prevalence by fecal culture ranged from 27.6% to 57.1%, depending on whether one, two or all three individual fecal samples in the positive fecal pool were culture positive (Table 1).

2. The seroprevalence study reported (official report to AF: October 2004) by M. Scott on behalf of Alberta Agriculture (Published: Scott et al., 2006) included 77 dairy herds selected randomly and serviced by Johne's disease accredited veterinarians, where complete samples and data were collected. A systematic random sampling protocol was used and samples were collected from the tail vein of 30 adult cows (≥2 lactations) and 7 first lactation cows per herd. Serum ELISA reported, at the animal-level, a prevalence of 9.1% (10.9% for cows with ≥2 lactations and 0.4% for 1st lactation cows) out of a total of 2819 adult dairy cows. The true overall adult animal-level prevalence of MAP infection was estimated to be 17.5%, while at the herd level the prevalence was 58.8% (Table 1).

The agroecological regions in Alberta have distinctive soil, climate and vegetation, and were shown to influence the risk of presentation of MAP at the herd level. In this manner, Parkland was almost 6 times at higher risk to have infected herds compared to Grassland. Other regions, such as Montane and Boreal Forest were at 4.7 and 1.4 times higher risk of having MAP-positive herds (Figure 2).
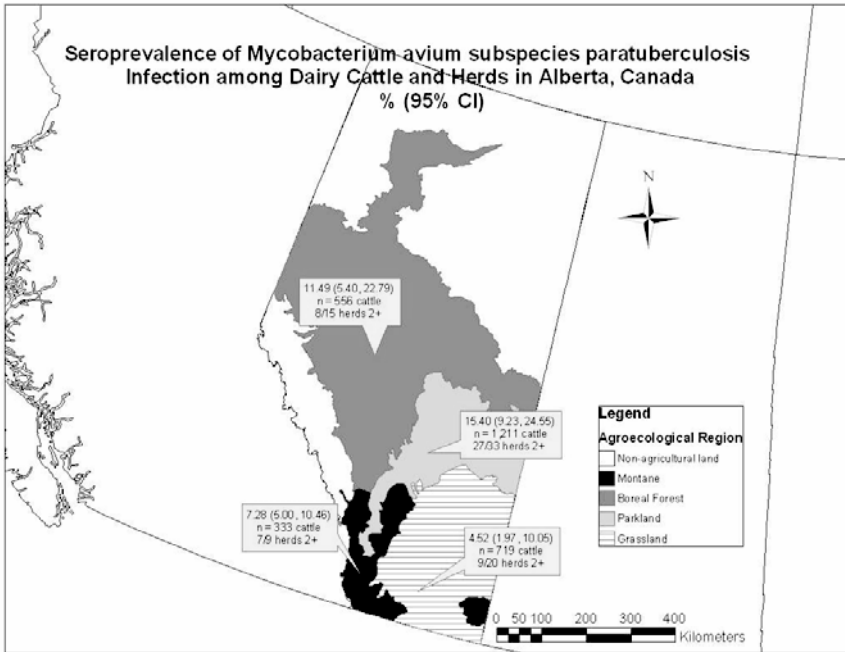
Figure 2. MAP seroprevalence in dairy herds in the major agro-ecological regions of Alberta. The parkland region was almost 6 times at higher risk to have infected herds compared to grasslands (Taken with permission from Scott MH, 2004).

It was concluded in this study that MAP has a high prevalence in individual dairy cattle, and that half of the dairy herds harbored MAP reactive animals.

The herd-level seroprevalence of MAP in dairy herds in Alberta (40.0 and 58.8%), appears higher than other regions of Canada, such as the Maritimes (16.7%)(VanLeeuwen et al., 2001), Ontario (30%)(Hendrick et al., 2005) and Saskatchewan (24.3%) (VanLeeuwen et al., 2005). However, variation within study design, demographics and diagnostic tests may account for part of these differences. At the animal-level, the true prevalence in Alberta from the two previous studies (8.1 and 17.5%) also appears higher as compared to the reports from the Maritimes (2.6%), Ontario (2.6%) and Saskatchewan (2.7%). Despite the variation between labs and tests, as well as the differences in the study design, it appears that the MAP is widely distributed among the geography of Alberta, with dairy herds and individual animals exhibiting greater seroprevalence compared to other provinces of Canada and other parts of North America (Hirst et al. 2004). Also, some agroecological regions have an influence in the prevalence of MAP among dairy cattle.

■ Programs and Projects in Alberta

Alberta Johne's Disease Working Group (AJDWG):

This is a joint industry and government body that has met for the past several years to advocate and develop effective Johne's disease prevention and control activities that will led to the reduction in the infectivity levels and eventual eradication of Johne's disease from provincial herds. At the centre of these activities is the Voluntary Johne's Herd Status Program (VJHSP). The AJDWG coordinated several meetings with its members during the past years in order to implement and evaluate the development of the status program. The AJDWG is the agency in charge of administering the program and assessing the submission of laboratory results and petitions for certification (Fig 3). AF administers the AJDWG and provides auditing, education, training and issuing of herd status certificates. Practicing veterinarians sit at the core of this program, acting in their client's best interest, whether it be to advise on testing for Johne's (and maintaining the confidential results), participating in the herd status program, or implementing control and/or better management practices where the disease is found to be prevalent.

The AJDWG in conjunction with the Food Safety Division (FSD) of AF designed and established a provincial veterinary accreditation program for veterinary practitioners in August 2001. This program provides specific information regarding Johne's disease in ruminants, the description of the VJHSP for cattle, the program logistics and most up-dated and relevant scientific information regarding the control of JD. Veterinary practitioners are required to obtain accreditation before they can participate in the VJHSP. Accreditation status is valid for three years.

The FSD of AF in conjunction with AJDWG conducts accreditation-training sessions for veterinary practitioners. During 2001, 2002, 2003 and winter of 2005, several accreditation-training sessions for veterinary practitioners were conducted across Alberta. Currently, 112 Alberta veterinarians are accredited to Voluntary Johne's Cattle Herd Status Program.

During the last AJDWG meeting (Jan'07), three industry sectors (Alberta Beef Producers, Alberta Milk, Alberta Sheep and Wool Commission) decided to join the Canadian Voluntary Johne's Disease Prevention and Control Program, which is an initiative proposed by the Canadian Animal Health Coalition (an industry-government national body). The AJDWG will conduct the discussion on the details, logistics and modifications for the adoption of this program by each particular industry in the province (A. Godkin, personal communication).

The Alberta Voluntary Johne's Herd Status Program (VJHSP):

This is an Alberta initiative in conjunction with AJWG initiated in September 2001 to reduce the likelihood of Johne's disease in Alberta cattle herds and certify tested herds that are not likely to be infected. The aim of the herd status program is to identify and categorize herds on the strength of apparent freedom from Johne's disease, to provide a simple system to communicate to cattle buyers the risk of Johne's disease infected animals and to stop the spread of this infection to non-infected cattle herds.

The multi-level voluntary program offers interested cattle producers the opportunity to test their herds using a pre-set protocol consisting of four levels. Accredited veterinarians who evaluate the Johne's disease status of their herds using specific sampling and testing protocols deliver this program. Each level represents an increase in confidence that the herd is free from JD (Figure 3).

If there is evidence of the disease, an accredited veterinarian and the producer will work together to develop a program suitable for each individual producer's needs. If there is no evidence of the disease, a program is developed to maintain this status or advance to the next level.

In order to participate in the herd status program, cattle producers must contact veterinarians accredited by the AJDWG. On enrollment, the participating veterinarian assigns each herd a code number in order to protect the confidentiality of producers. The veterinarian collects samples (blood-feces) from test eligible cattle and submits to a USDA certified laboratory for Johne's testing. To obtain certification, the individual animals tested eligible should be serum ELISA negative or confirmed negative on fecal culture. In order for a farm to escalate to other levels it should test negative on individual fecal cultures and on strategic pooled fecal samples as it progresses into higher levels (Figure 3). Herd confidentiality is maintained up to the point where certificates are issued stating that they have successfully met conditions for a given level. The accredited veterinarian applies for herd status certification by submitting documentation on the producer's behalf to the AJDWG. AF issues a Herd Status Certificate to the producer.

Currently, the Voluntary Johne's Disease Herd Status Program in Alberta has 16 dairy herds enrolled, with 6 of them renewed in different levels of the program (Level I: 4 herds, Level II: 1 herd; Level IV: 1 herd).

On the dairy side, the VJHSP has 21 herds enrolled, with 7 of them renewed in different levels of the program (Level I: 5 herds; Level III: 1 herds; Level IV: 1 herd)
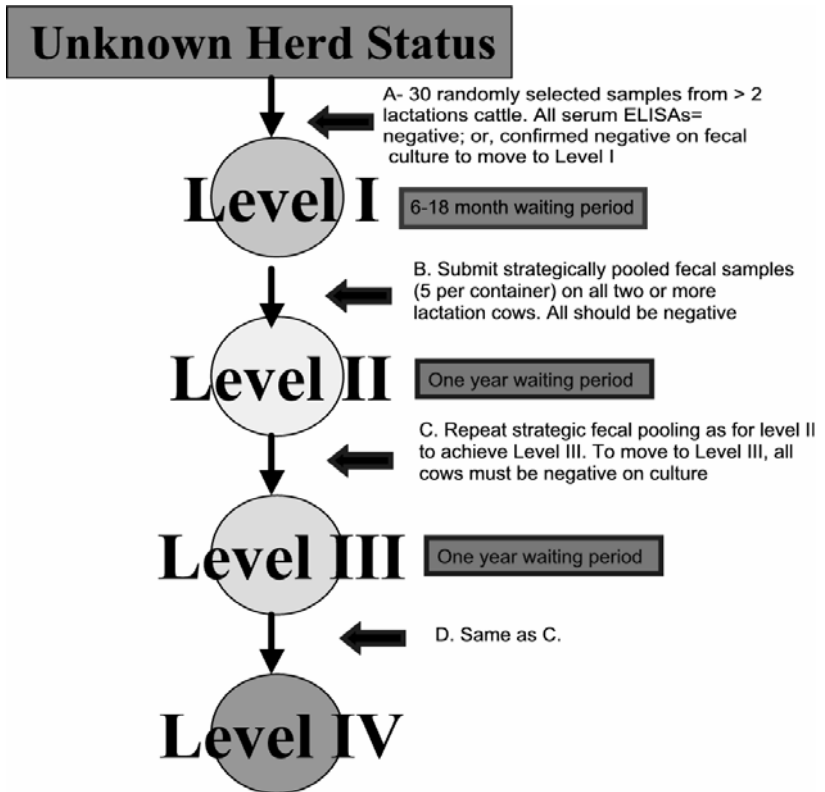
Figure 3. Description of the four-levels of the Voluntary Johne's Disease Herd Status Program. Herd owners can maintain herd status level with annual ELISA testing of 30 random samples from cattle second lactation or greater. All cows must be negative ELISA or confirmed negative on fecal culture

CanWest DHI Johne's Disease Prevention Project:

The CanWest DHI project was initiated in Ontario two years ago and last year it was extended to the western provinces. Several industry organizations, government agencies and academia are the project partners. This project began in Alberta on March 2006 to provide assistance to veterinarians and dairy producers to develop sound calf rearing programs that help prevent the spread of JD.

The rationale for this project was based on the understanding of the epidemiology and transmission of MAP. The most common mode of transmission of MAP is trough freed and water contaminated with manure from infected animals. Fecal shedding from clinically infected animals may reach more than 500 billion organisms per day. Also, up to 35% of cows with

advanced clinical disease may shed MAP in their milk with newborn calves becoming infected through colostrum. As a result, the environment can become heavily contaminated and most animals on these farms have a high risk of exposure to MAP and to become infected, particularly those less than six months of age. Also, MAP is resistant to environmental degradation and to many disinfectants, survives in stagnant water, manure, and deep soil, and withstands freezing at -14°C for up to a year. Therefore, it is essential to reduce exposure of younger calves to contaminated manure, dirt, and milk and to separate them from the dam as soon as possible after birth.

This project is executed through trained veterinarians who conduct risk assessments and evaluate the potential financial impact of JD in a particular herd. The veterinarian provides best management recommendations and available testing options regarding JD to dairy producers. Currently, 32 veterinarians and 75 dairy producers are enrolled in the project. The project reimburses each veterinary practitioner $200 towards the cost of the training and each producer receives a $400 DHI credit to help them fund JD investigation.

The procedure to enroll in the project is initiated by the trained veterinarian, who submits information regarding a particular producer interested in joining the project. The producer should sign a consent form to allow testing against MAP in the next set of milk samples that are routinely tested for mastitis by DHI. After having the results back, the veterinarian on the producer's farm conducts a risk assessment and a report is submitted to the producer with recommendations and suggested changes.

To date, out of 5201 cows tested, 21 (1.5%) were positive, while 10 (0.2%) are suspect and 5098 (98%) were negative at milk ELISA. At the herd level, 23 (39%) of 59 herds had a least one positive cow.

In Ontario, a project is in progress to evaluate the compliance of producers to better management recommendations given by practitioners and the reasons for not meeting those recommendations. This project will extent to other provinces of Canada, including Alberta. A long-term goal of this project is to evaluate the differences in prevalence among herds adopting better management practices and those that did not.
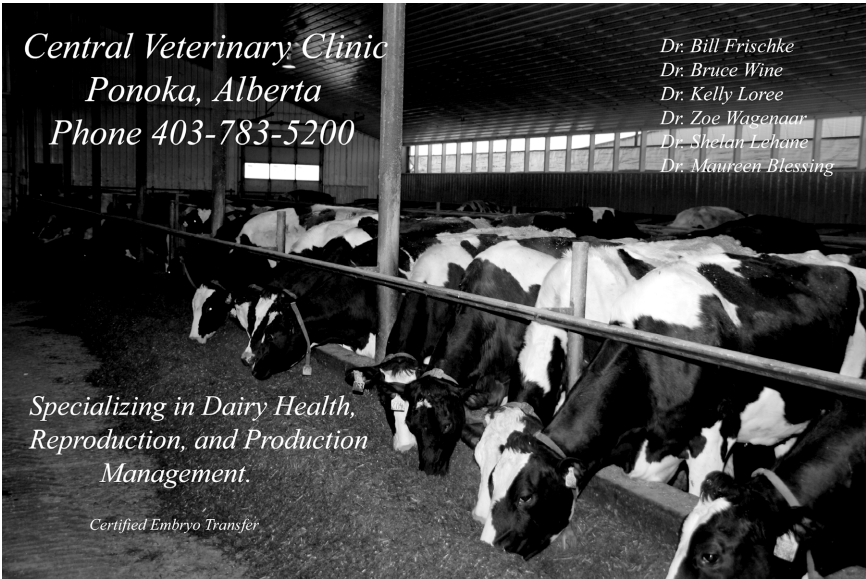
Summary

It is important for producers to learn about the risks and to implement best management practices and biosecurity to reduce the risk of infection to newborn calves. Raising calves in a way that reduces their exposure to MAP increases producers' odds of keeping replacements healthy and infection-free. If a producer can effectively prevent infection in his replacement stock, over a period of time JD may be eliminated from the farm. A JD calf-rearing

program has the added benefit of limiting other calf-hood disease and deaths caused by different manure-borne pathogens. Testing at certain intervals is another tool to determine the herd status and to evaluate over time the result of current and future changes on management practices.

■ References

Barrow G.I., Felttham R.K.A. (eds). 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge University Press, pp 92-93.

Hirst H.L., Garry F.B., Morley P.S., et al. 2004. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity. J Am Vet Med Assoc 225:97-101.

Hendrick S, Duffield T, Leslie K, Lissemore K, Archambault M, Kelton D. 2005. The prevalence of milk and serum antibodies to *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds in Ontario. Can Vet J 46:1126-1129.

Humphry R.W., Stott A.W., Adams, C, Gunn G.J. 2006. A model of the relationship between the epidemiology of Johne's disease and the environment in sucker-beef herds. Vet J 172:432-445.

Ollis G, VanderKop M. 2000. Johne's disease surveillance: A baseline study in beef cattle. Animal Health Forum 5(2).

Radostits O.M., Gay O.C., Blood D.C., Hinchcliff K.W. 2000. Veterinary Medicine A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. Harcourt Publishers Ltd., London.

Scott, M.H. 2004. Prevalence and risk factors for Johne's disease (and other infectious production-limiting diseases) in Alberta beef cow-calf and dairy herds Alberta Agriculture and Food. Final Report, Edmonton.

Scott M.H, Sorensen O, Wu J, Chow E, Manninen K, VanLeeuwen J.A. 2006. Seroprevalence of *Mycobaterium avium* subspecies *paratuberculosis*, Neospora caninum, Bovine leukemia virus, and Bovine viral diarrhea virus infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity. Can Vet J 47:981-991.

Sorensen O, Rawluck S, Wu J, Manninen K, Ollis G. 2003. *Mycobacterium paratuberculosis* in dairy herds in Alberta. Can Vet J 44:221-226.

VanLeeuwen J.A., Keefe G.P., Tremblay R, Power C and Wichtel J. 2001. Seroprevalence of infection with *Mycobaterium avium* subspecies *paratuberculosis*, bovine leukemia virus, and bovine viral diarrhea virus in Maritime Canada dairy cattle. Can Vet J 42:193-198.

VanLeeuwen J.A., Forsythe L, Tiwari A, Chartier R. 2005. Seroprevalence of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* in dairy cattle in Saskatchewan. Can Vet J 46:56-58.

Wu J, Ollis G. 2001. A survey to estimate the seroprevalence of Johne's disease in Alberta Beef herds. Food Safety Division, Alberta Agriculture and Food internal report, Edmonton.