Changes in management practices and apparent prevalence on Canadian dairy farms participating in a voluntary risk assessment-based Johne's disease control program

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ABSTRACT

The objectives of this study were (1) to describe the change in *Mycobacterium avium* ssp. *paratuberculosis* (MAP) antibody milk ELISA-positive prevalence in Canadian dairy herds that participated in a risk assessment (RA)-based Johne's disease (JD) control program; (2) to describe the distribution of so-called high-risk management practices on Canadian dairy farms; and (3) to assess if compliance with selected recommendations translated into changes in the scores of associated RA questions. In Ontario and western Canada, 226 herds voluntarily participated in a RA-based JD control program for several years. In 2005–2007, a pre-visit survey, RA, and MAP-antibody milk ELISA of the entire milking herd were conducted. Therefore, the interpretation of the results of this study is strictly for the MAP-antibody milk ELISA status of cows or herds, because no culture of MAP (of fecal or environmental samples) was conducted due to economic restrictions. In early 2008, a telephone interview was used to determine compliance with recommended management changes after the first RA. In 2008–2009, a second RA and another whole-herd MAP antibody milk ELISA were performed. At both herd tests, about 35% of the farms had at least one MAP-antibody milk ELISA-positive cow, classifying them as a MAP-antibody milk ELISA-positive herd. However, 28.8% of herds had changed their MAP-antibody milk ELISA status between the 2 tests, demonstrating that a single herd test was insufficient to determine the long-term MAP-antibody ELISA status of a herd. The average within-herd MAP-antibody milk ELISA-positive prevalence changed from 5.4 to 4.2% over the study period, but management practices did not change much throughout

the 2- to 3-yr period and were similar to those reported in other parts of North America. The overall RA scores decreased at the second RA, in particular for management practices in the calving and preweaned calf area, and when herds were test-positive at the first test. This was not surprising, because many of the recommendations at the first RA focused on these management areas and compliance with some recommended farm-specific management practices in this area might be linked to reduced scores for associated RA questions. In conclusion, the participating farms did, on average, decrease their within-herd MAP-antibody milk ELISA positive-prevalence and RA total scores. Changes in RA scores could be linked to improved management practices, indicating that the RA questions appropriately reflected management practices. Some herds changed their MAP-antibody milk ELISA status between tests, which underlines that a current test of the entire milking herd is necessary to determine the present MAP-antibody milk ELISA status of a dairy herd.

Key words: paratuberculosis, risk assessment, cow, Johne's disease

INTRODUCTION

Johne's disease (JD) is the clinical manifestation of a chronic disease of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). Although the majority of infected cows are subclinical, the infection (paratuberculosis) can lead to decreased milk production and premature culling of infected animals (Hasonova and Pavlik, 2006). The pathogen is primarily transmitted fecal-orally, but it is also secreted into colostrum and milk. Calves seem to have the highest susceptibility to infection (Windsor and Whittington, 2010). Currently available diagnostic tests are imperfect, meaning that many infected and possibly MAP-shedding animals go undetected. Therefore, the implementation of best management practices to reduce exposure of calves to MAP is assumed to be crucial for paratuber-

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culosis and JD control on dairy farms. Risk assessment (RA)-based JD control programs evaluate and score current management practices for their potential to spread MAP between and within farms. The questions comprising the RA used in this study are based upon a combination of expert opinion, biological plausibility, and anecdotal reports. The component parts of the RA have not been formally evaluated, primarily because of the long lag period between initial infection and diagnosis of infection or clinical disease. Furthermore, it is not clear if the RA accurately reflects management practices on the farm, or which management practices are used in Canadian dairy herds, as differences may exist compared with other countries due to herd size, climate, and so on. This knowledge could help to differentiate between management practices on farms with and without MAP-infected animals, which will ultimately allow us to refine our understanding about which management practices are most important for preventing the spread of MAP on dairy farms. Because the culture of MAP of individual cows in this study was cost-prohibitive, a MAP-antibody milk ELISA was used.

The objectives of this study were (1) to describe the distribution of management practices associated with the MAP-antibody ELISA prevalence on Canadian dairy farms; (2) to assess if compliance with selected recommendations translated into changes in the RA scores; and (3) to describe the change in MAP-antibody ELISA-positive prevalence in Canadian dairy herds that participated in a JD control program.

MATERIALS AND METHODS

In 2005, a pilot study for a voluntary RA-based JD control program was initiated by the Ontario Ministry of Agriculture, Food and Rural Affairs, and CanWest DHI. The program was funded by Canadian Adaptation Council, a granting agency of the federal and provincial governments. Eighty Ontario dairy herds that had known or suspected JD cases in previous years and were enrolled in milk recording programs with CanWest DHI were recruited into the program by 80 practicing veterinarians. The program consisted of a previsit survey about general farm management practices (e.g., manure handling, biosecurity protocols), an RA conducted on farm by the herd veterinarian, and a MAP-antibody milk ELISA of all milking cows done on one DHI test-day. The RA was adapted from an RA developed in the United States by Rossiter et al. (2002). Table 1 lists the RA questions, their abbreviations, and the range of possible scores. The higher the score, the greater the attributed risk of spreading MAP through a given management practice. The scores were

summarized by section and an overall herd score was calculated.

The program was extended in 2006 and 2007 to additional dairy herds in Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia. Dairy producers were invited to participate via articles in producer magazines and veterinary newsletters and did not have to be CanWest DHI members. The previsit survey and RA were slightly modified. A question about the feeding of frozen colostrum (CA16) was added to the RA and questions about the herd's JD testing and history were removed from the RA and incorporated into the previsit survey. In total, 627 herds participated in the project (including the 80 herds in the pilot study). In addition, as part of the program, 182 veterinarians in the 5 provinces were trained to conduct the RA. Training was conducted in Ontario by 2 veterinarians from the Ontario Ministry of Agriculture, Food and Rural Affairs or by a designated provincial veterinary trainer in each of the 4 other provinces to minimize interobserver variability. However, no formal evaluation of interobserver agreement was conducted.

In late 2007, a formal follow-up study of herds participating in the original project was initiated. To enable access to electronic production records, only herds that were CanWest DHI customers and had tested their entire milking herd were eligible to participate in this follow-up study. To assess whether changes in management practices were associated with fewer test-positive first-lactation cows, all first-lactation cows were required to be tested at the first test (test 1). However, some herds were found to have tested only a few or none of their first-lactation animals, and therefore, herds were excluded if fewer than 20% of tested cows were first-lactation animals. After removal of 12 herds that had ceased dairy farming, 499 herds in Ontario and western Canada were invited to participate in the follow-up study in December 2007.

The follow-up study consisted of a telephone survey (spring 2008), a second on-farm RA, and MAP-antibody milk ELISA of the entire milking herd (November 2008 to November 2009; Figure 1). During the telephone interview, producers were asked about their compliance with recommendations made by their veterinarian at the conclusion of the initial RA (Sorge et al., 2010a). The second or follow-up RA was targeted to be conducted at least 2 yr after the first RA. The second MAP-antibody ELISA of the entire milking herd (test 2) was scheduled at least 2.5 yr after the first herd test and RA (Figure 1). This interval was chosen to allow for a lag period between the recording of management practices to which new heifer calves would be exposed and their entrance into the milking herd, allowing for their testing with the MAP-antibody ELISA.

Table 1. Questions composing the risk assessment (RA) questionnaire¹

Area	Question
Calving area (RAS: 0, 3, 5, 7, 10)	
CA1	How often is more than one cow present in a maternity boxstall at a time?
CA2	How often is new bedding added to the box stall and how does it look today?
CA3	If you kneel down on the bedding in the calving pen today for 25 s, is your knee wet? (RAS: 2, 8)
CA4	How often is a lame, sick, or other special-needs cow in the calving stall?
CA5	Have Johne's disease (JD) clinical or test-positive animals calved in the same area as noninfected cows, before today?
CA6	Currently, do you have specific strategies for dealing with JD clinical or test positive cows at calving? (Is anything different done with these cows?) (RAS: 2, 8)
CA7	How often are calves born in tie-stalls, freestalls, pasture, or other cow contact areas?
CA8	How clean are most of the cow's udders and hind legs in the calving area?
CA9	Are udders clipped and cleaned before calving?
CA10	Are calves removed from the cow within 30 min of birth?
CA11	Are calves allowed to nurse their mother or other cows?
CA12	How old are calves when separated from mothers?
CA13	What percentage of all calves are fed at least 4 L of colostrum (if Holstein calves) within 6 h of birth?
CA14	How often is colostrum that comes from more than 1 cow (pooled colostrum) fed to a heifer calf?
CA15	How often have heifer calves been fed frozen colostrum from another cow (not their own mother) in the last year? (RAS: 0, 3, 5, 10)
CA16	Has the colostrum fed to heifer calves been from low-risk JD mothers? (RAS: 0, 2, 5, 7, 10)
Preweaned calves (RAS: 0, 3, 5, 7, 10)	
PW1	Does the milk that is fed to heifer calves come from low-risk cows? (RAS: 0, 2, 5, 7, 10)
PW2	Is pooled whole milk fed to heifer calves? (e.g., milk that is collected from the bulk tank, the milk line, the receiver jar, or multiple cows each milking)
PW3	Is nonsaleable milk (fresh, mastitis, or treated cow milk) fed to heifer calves?
PW4	If nonsaleable (waste) milk is fed to heifer calves, is it pasteurized first? (RAS: 1, 8)
PW5	Are calf bottles and pails washed (not just rinsed) with soap and water daily? (RAS: 1, 3, 5; for pails and bottles each)
PW6	Does the same bottle or pail go to the same calf each day? (RAS: 1, 3, 5; for pails and bottles each)
PW7	Do heifer calves have contact, in any way or at any time, with cows or cow manure after 1 d of age?
PW8	Is calf milk, feed, or water contaminated with cow manure?
PW9	Do people from this farm walk among calves after contact with cow manure, without cleaning or changing their boots?
Weaned calves (RAS: 0, 1, 2, 3, 5)	
W1	Do heifers have contact with cows or cow manure?
W2	Is feed or water contaminated with cow manure?
W3	Is feed (including leftover feed), water, or housing shared with cows?
W4	Is feeding equipment used to feed heifers contaminated with manure?
W5	Do heifers share (at the same time) or graze (not at the same time) the same pasture with cows (dry or milking)?
W6	Is manure spread on any forage that will be grazed by, or harvested for, heifers in the same season?
Breeding-age heifers (RAS: 0, 1, 2, 3, 4)	
BH1	Do breeding-age heifers have contact with cows or cow manure?
BH2	Is feed or water contaminated with cow manure?
BH3	Is feed (including leftover feed), water, or housing shared with cows?
BH4	Is feeding equipment used to feed breeding age heifers contaminated with manure?
BH5	Do breeding age heifers share pasture with cows?
BH6	Is manure spread on any forage that will be grazed by, or harvested for, breeding age heifers in the same season?
Cows (RAS: 0, 1, 2, 3, 4)	
C1	Are feeders or waterers contaminated with manure?
C2	Are feeding equipment or feed storage areas contaminated with manure?
C3	Do cows have access to manure storage areas or run-off?
C4	Is manure spread on forage grazed by, or harvested for, cows in the same season?
C5	Milking cows hygiene score (RAS: 0, 1, 2)
C6	Close-up dry cows (within 3 weeks of calving) hygiene score (RAS: 0, 1, 2)

¹Unless otherwise stated, the answer categories to the questions in each section had possible RA scores (RAS) as stated at the beginning of the section.

Throughout the study, the same MAP-antibody milk ELISA was used (Parachek, Prionics, Zurich, Switzerland). The test has a reported individual animal sensi-

tivity of 28.9% and specificity of 99.7% compared with fecal culture (Collins et al., 2005). An animal with an optical density of ≤ 0.1 was defined as test-negative on

Figure 1. Timeline and study design.

the test-day, and an animal with an optical density of >0.1 was test-positive. For the purpose of this study, we assumed that a positive MAP-antibody ELISA reflected a MAP infection of that animal. In addition to the MAP-antibody ELISA results, CanWest DHI provided each cow's production data from that test-day, as well as herd-level information about parity distribution, average milk production by parity, SCC, and an inventory of cows on the farm. Because of the limited number of cows in lactation ≥ 4 , these cows were collapsed into a single parity group.

Some herds ($n = 8$) had decided to split the MAP-antibody ELISA testing of their milking herd over multiple test-days. For those herds, the test results of the cows were combined and a central test-day was selected as the time point for determining herd status. For cows from these herds that had more than one test in 1 yr, their first MAP-antibody ELISA was chosen to be included in the analysis. None of the test-positive cows had more than one test. The MAP-antibody ELISA herd status was classified as either test-positive or test-negative. A test-positive herd was defined as a herd that had at least 1 MAP-antibody milk ELISA-positive cow.

Statistical analysis was conducted in SAS 9.2 (SAS Institute Inc., Cary, NC) and the significance level was set at $\alpha = 0.05$. The distribution of management practices potentially associated with MAP transmission on Canadian dairy farms, as recorded on the previsit survey and first RA, was described using appropriate descriptive statistics. The answer categories of RA or previsit survey questions were described with proportions (PROC FREQ). Questions with normal or non-normal, continuous answers were described using either mean and standard deviation, or median and interquartile range (IQR), as well as the range of values of observations, respectively.

A latent variable approach was used to assess associations between RA scores (RAS) and other continuous variables, and to compute differences in RAS between

the second and first RA. Correlation was assessed using a nonparametric Spearman correlation, and changes in RAS were compared between herds that were either test-negative or test-positive at test 1 using a Mann-Whitney U-test.

The MAP-antibody milk ELISA-positive prevalence and MAP-antibody milk ELISA herd status were assessed at the herd level. Individual cow records were used to describe the apparent prevalence by parity within test-positive herds. The apparent within-herd prevalence at both tests, the change in prevalence in test-positive herds that had culled test-positive cows and those that had not culled cows, was compared with a Mann-Whitney U-test.

The within-herd MAP-antibody milk ELISA-positive prevalence by parity was compared between test 1 and test 2 using the Sign test. The difference in apparent prevalence in first-lactation cows was additionally correlated with the difference in RAS between the first and second RA using the nonparametric Spearman correlation.

During the telephone survey, the producers were asked whether they had implemented farm-specific recommendations. The association between compliance (yes/no) with the 10 most frequently made recommendations (Sorge et al., 2010a) and changes in RAS of related RA questions was assessed using a Mann-Whitney U-test. One recommendation (to test the dry cows for MAP infection) did not have corresponding questions in the RA and therefore was not included in this analysis. The RAS were again used in a latent variable approach.

RESULTS AND DISCUSSION

Initially, 238 dairy producers of 499 contacted (48%) agreed to participate in the follow-up study and completed the telephone survey. However, ultimately, only 226 herds completed a second whole-herd milk test (test 2), and only 207 of these also completed a

second RA with their herd veterinarian in 2008–2009. The 226 herds were similar to the 499 herds originally contacted (Table 2) and represented about 5% of CanWest DHI members in each province. Compared with the population of all CanWest DHI herds, these herds were, on average, slightly smaller and had higher milk production. Similarly, Raizman et al. (2006) found that herds enrolled in the JD control program in Minnesota produced more milk than the average of all Minnesota DHIA herds. Therefore, it might be inferred that the participating producers represented more progressive and informed dairy farmers and caution is warranted in the extrapolation of results reported here to all dairy farmers in Canada.

The management practices used on farm were recorded using the previsit survey and both RA. Only minor changes were observed between the RAS distribution of the first RA in 2005–2007 and the second RA in 2008–2009. Therefore, the distribution of selected management practices presented herein is based on the 2005–2007 RA unless otherwise stated. Detailed information on the distribution of management practices assessed with the previsit survey (Sorge et al., 2010b) and the RA in 2005–2007 and 2008–2009 (Sorge et al., 2010c) are published online. The average time between test 1 and test 2 was 33.4 ± 4.5 mo (range: 22 to 48 mo), and the average herd in the study increased in size by 6 ± 12.7 cows (range: –36 to 103 cows) over that period.

General Management Practices

The observed distribution of housing systems was comparable to that reported for dairy herds in the United States (USDA, 2009). The majority of farms housed their milking cows in tie-stall (55%) or freestall barns (42%). Forty-nine of the farms (21%) did not purchase any cattle in the 20 yr before the first RA. Of the herds that purchased mature cows ($n = 175$, 77%), most did not have an entry protocol for those cows. However, although 31% of farms in the United States isolated newly purchased cows (USDA, 2010), 91% of the study farms did not isolate purchased animals upon arrival. Furthermore, although the farmers in this study were inclined to ask about the individual cow's SCC history (62%), they did not ask about the herd's SCC (58%) or vaccination protocol (56%) of the cow's herd of origin. Questions about JD history (e.g., test results) of the cow (13%) or farm of origin (15%) were only asked by a minority of producers, despite their awareness of JD.

None of the farms had vaccinated calves against JD, which is not surprising considering that there is no licensed JD vaccine in Canada and it could only be

Table 2. Demographics of herds contacted and enrolled in follow-up study as well as CanWest DHI customers by province, including median herd size, 305-d milk production (M305), and percentage of test-positive herds (i.e., herds with ≥ 1 *Mycobacterium avium* ssp. *paratuberculosis*-antibody milk ELISA-positive cow during the 2005–2007 test period)

Province	Contacted				Enrolled				CanWest DHI clients				
	Herds, n	Herd size	M305	Positive, %	Herds, n	Herd size	M305	Positive, %	Response rate, %	Herds, n	Herd size	M305	Enrolled, %
Ontario	359	55	9,356	37	173	64	9,161	36	48	3,196	72	8,407	5
Manitoba	36	87	9,248	53	12	121	9,749	50	33	222	121	8,365	5
Saskatchewan	30	102	9,972	27	9	130	9,662	0	30	140	137	8,778	6
Alberta	34	81	10,233	38	16	96	10,389	25	47	468	127	9,164	3
British Columbia	40	89	9,858	43	16	102	10,359	31	40	327	132	7,421	5
Total	499			38 ¹	226			35 ¹	45 ¹	4,353			5 ¹

¹Average.

¹Average.

obtained using Emergency Drug Release services of the federal government.

Calving Facilities

The calving area has been previously recognized as a great risk area of transmission of MAP and new infections of newborn calves (Benedictus et al., 2008). Therefore, the most commonly made recommendations predominantly focused on improved hygiene of calving area and rapid removal of the newborn calf to reduce the exposure of calves to fecal material, colostrum, or milk of potentially infectious adult cows (Sorge et al., 2010a).

Although in 2005–2007, the majority of participating farms (60%) never calved high-risk JD cows (i.e., clinical or test-positive) in the same maternity pen as the rest of the herd, 26% and 10% of farms had calved high-risk cows on rare occasions (RAS = 3) or routinely in the same maternity area as the rest of the herd (RAS = 10), respectively. Nevertheless, between the 2 RA, the percentage of producers who had a strategy for calving test-positive cows increased from 41 to 70%. This was also reflected by the fact that in 2008–2009, most farmers (74%) calved MAP test-positive or “suspect” cows in specific areas outside the maternity pen, which could indicate that the producers became more conscious of potential MAP transmission pathways on their farms after participating in this program.

Newborn Calves

In 2005–2007, calves were seldom removed from the calving environment within 30 min after birth (10%, RAS = 0). Usually, producers removed the calves within 3 (42%, RAS = 3) to 12 h (37%, RAS = 5) after birth, and although many producers did not allow the calves to suckle their dam (18%, RAS = 0), roughly the same number of producers (17%, RAS = 10) always allowed the calves to suckle. However, by 2008–2009, farmers were removing the calves sooner (20%, RAS = 0) and fewer herds allowed suckling for all calves (10%, RAS = 10). This compliance with the recommended rapid removal of newborn calves from the maternity area was associated with significantly lower scores at the second RA for questions about the frequency of removal of calves within 30 min of birth (CA10; $P = 0.0523$), whether calves were allowed to nurse their dam (CA11; $P = 0.0130$), and how old the calves were when they were separated from the dam (CA12; $P = 0.0021$; Table 3).

The colostrum fed on the study farms predominantly came from the calf's dam only (RAS = 0; 58%), and frozen colostrum was rarely used. The source of co-

lostrum was test-negative cows (44%), unknown-status heifers (8%), or unknown-status cows (39%). It was encouraging to note that the number of farms that knowingly fed their calves colostrum from test-positive cows decreased from 4 farms (2%) in 2005–2007 to none in 2008–2009. Likewise, the number of farms that were feeding only artificial colostrum to calves increased from 15 (7%) to 24 (11%) in the same period. In contrast, in the US dairy herd study, approximately 5% of dairy farms continued to feed colostrum from known MAP-antibody test-positive cows to calves (USDA, 2010). Compliance with the recommendation to feed low-risk colostrum tended to be associated with a decreased RAS to question CA13, which assessed the amount of colostrum fed to calves (Table 3). Likewise, the RAS of the same question was significantly reduced when the producer reported to have increased the amount of colostrum fed to calves as suggested after the first RA ($P = 0.009$; Table 3).

An observation that has to be interpreted carefully is that the majority of producers (77%) in this study indicated in 2008–2009 that they provided colostrum from test-negative cows only. Less than half of the herds (30%) had tested their milking herd for MAP-antibodies within the 2.5 yr of the follow-up period. Therefore, the majority of farmers were relying on results from the single herd test done in 2005–2007. This could have been misleading because some herds became MAP-antibody milk ELISA-positive at test 2. It is important to note that at test 2, given the long timespan between the 2 MAP-antibody milk ELISA, not only were approximately 60% of the herd different animals due to a normal turnover rate, but most herds had purchased cattle of unknown status (possibly introducing MAP-infected animals to the farm). Furthermore, the MAP-antibody milk ELISA has a low sensitivity and imperfect specificity, which could have resulted in false-negative or false-positive test results at either test. Therefore, the likelihood and extent of a MAP infection of a dairy herd can only be estimated with a current herd test, although it has to be recognized that a positive MAP-antibody milk ELISA does not necessarily equal MAP infection.

Prewaned Calves

Once calves were removed from their dams, the milk-fed calves were most commonly housed outside in individual hutches (36%). In contrast, the herds in Quebec mostly housed their calves inside, and only 8% of farms housed their preweaned calves outdoors (Vasseur et al., 2010).

Although the majority of producers always fed pooled milk to their calves (e.g., bulk tank milk, RAS

Table 3. Association between compliance with the 10 most commonly made recommendations and changes in associated risk assessment (RA) question scores

Recommendation	n	RA question ¹	P-value
Remove newborn calves quickly (before standing) from maternity pen	123	CA10	0.0523
		CA11	0.0130
		CA12	0.0021
Feed low-risk colostrum from test-negative cows or heifers	109	CA11	0.5829
		CA13	0.0582
		CA14	0.2192
		CA15	0.3554
		CA16	0.8785
Feed low-risk milk to calves	69	PW1	0.4372
		PW2	0.2337
		PW3	0.5039
		PW8	0.0811
Feed more colostrum to calves	69	CA13	0.0009
Separate the newborn calf from cows, create a mini-pen or use a calf-box	67	CA10	0.1074
		CA11	0.7946
		CA12	0.1402
Do not feed nonsaleable milk ("waste milk") to heifer calves	62	PW3	0.3888
		PW4	0.6568
Do not walk through feed or calf pens with cow manure on boots (rinse boots or have separate pair for calf area)	60	PW7	0.6323
		PW8	0.6610
		PW9	0.1081
		W1	0.3683
		W2	0.3128
Wash pails and bottles for calves with soap and water daily	60	PW5B	0.3912
		PW5P	0.2102
		PW6B	0.6109
		PW6P	0.4725
Identify test-positive and suspect cows as well as their relatives and offspring	58	CA5	0.3188
		CA6	0.6634
Do not calve Johne's disease clinical or test-positive/suspect cows in the same area as test-negative cows	45	CA5	0.3567
		CA6	0.9354

¹See Table 1 for full RA question.

= 10, in 2005–2007: 66%; in 2008–2009: 59%), more farms fed milk replacer in 2008–2009 (31%) than in 2005–2007 (19%). On 51 farms (23%), the calves were always (RAS = 10) fed nonsaleable milk (i.e., fresh, mastitis, or treated cow milk). However, in 2005–2007, only 5 operations (2%) pasteurized nonsaleable milk before feeding it to calves, whereas in 2008–2009, 11 farms (5%) were pasteurizing the nonsaleable milk. When producers indicated that they had complied with the recommendation to feed low-risk milk to calves, the quality of the milk fed tended to be improved; that is, the RAS for that question tended to be lower at the second RA ($P = 0.0811$, Table 3). Furthermore, the proportion of operations using pasteurization in 2008–2009 was greater than in herds studied in Quebec and the United States, where none (Vasseur et al., 2010) or only 2.6% (USDA, 2010) of farms fed pasteurized milk, respectively.

The calves were fed milk from either bottles or pails, which were sanitized daily on 64 and 42% of farms, respectively, but did not go back to the same calf in most instances (82 and 61%, respectively). By 2008–2009, farms had slightly improved their cleaning routines; at

that time, 73 and 52% of farms cleaned the calf bottles and pails daily, respectively. However, this improvement was not associated with significantly improved RAS (Table 3).

Although the preweaned calves were estimated to have contact with cows or cow manure only rarely after the first day of life (21%, RAS >0), 33% of farmers routinely walked (RAS = 8) through calf pens without cleaning their boots of cow manure, which could expose calves to infectious cow manure. Only 38% of producers (RAS = 2) consciously avoided walking among calves without previously cleaning cow manure from their boots. The prevalence of this behavior did not change in the second RA.

Change of RAS

Although farmers could have reported changes in management practices to receive lower scores in the RA, participating veterinarians reported, during a survey conducted before the second RA was performed, that their clients had implemented at least some of the management practices recommended after the first RA

(Sorge et al., 2010d). The low number of statistically associated changes in RAS of individual questions and compliance (Table 3) is likely because the producers did not fully comply with the recommendations despite stating otherwise at the telephone survey, or because of insufficient statistical power to detect the difference.

The observed average scores for each management area or section of the first and second RA are presented in Table 4. The section RAS were positively correlated throughout the farm ($P \leq 0.06$); the higher the initial RAS, the greater the improvement (i.e., decrease in RAS at the second RA; $P < 0.001$). In particular, the RAS for questions of the calving area and preweaned calf husbandry practices were significantly improved. The majority of producers had lower and improved overall RAS for these 2 areas (66.7 and 64.5%, respectively) in 2008–2009. More than half of the producers (51.3%) dropped at least 20 points and 20.6% herds dropped to ≤ 60 points at their total RAS in 2008–2009. Nevertheless, on a few farms (20%), the RAS totals increased by 20 points. Raizman et al. (2006) and Wells et al. (2008) similarly observed decreased RA scores between 2 sequential RA, indicating that the producers were implementing recommended management practices.

Although herd size was generally not correlated with the summary scores of the different areas in 2005–2007 ($P > 0.6$), larger herds had lower RAS for preweaned calves ($r = -0.13$; $P = 0.0724$), weaned calves ($r = -0.19$; $P = 0.0087$), and breeding-age heifers ($r = -0.17$; $P = 0.0226$) at the second RA in 2008–2009.

The MAP-antibody milk ELISA herd status at test 1 was not associated with total scores or development of RAS area sums ($P > 0.07$). However, farms with MAP-antibody milk ELISA-positive cows at test 1, based on the RAS of individual questions, improved hygiene around calving, removed calves sooner, and were more likely to feed low-risk colostrum or milk and to separate their young livestock from adult cows by 2008–2009 compared with herds that did not have MAP-antibody

milk ELISA-positive cows in 2005–2007. This is not surprising, because most of the recommendations made in 2005–2007 focused on the calving area and preweaned heifer management, and test-positive herds received more recommendations than test-negative herds in 2005–2007 (Sorge et al., 2010a). Contrary, test-negative herds improved their overall cow cleanliness (RA questions CA8, CA9, C5, C6) more than MAP-antibody milk ELISA-positive herds ($P \leq 0.0217$).

Change in Apparent Prevalence

Table 5 shows the change in apparent prevalence at the herd level between test 1 and test 2, and Table 6 shows the apparent prevalence stratified by lactation, within population and within herd level, at both tests. When the cut-point for classifying a herd was changed from 1 to 2 test-positive cows, the herd-level MAP-antibody milk ELISA prevalence changed from 34.5 to 20.4% at test 1, and from 35.8 to 20.4% at test 2 (Table 6). The average age of test-positive cows ($P = 0.3387$) and distribution of parities ($P = 0.1391$) were similar at test 1 and test 2.

The majority of herds either remained test-negative or had a lower within-herd MAP-antibody milk ELISA positive prevalence at test 2 (Table 5; $P < 0.0001$). The apparent within-herd prevalence among all 226 herds decreased on average by $0.34 \pm 2.9\%$ (median = 0; IQR = -0.33 ; 0), which was not significant ($P > 0.4$). The apparent within-herd prevalence of MAP-antibody milk ELISA-positive herds decreased by $2.2 \pm 3.9\%$ (median = -2.0 ; IQR = -4.2 ; -0.2 ; $P < 0.0001$) between the 2 tests; the changes in within-herd prevalence ranged from -18.0 to 7.8% . The observed decrease is similar to that observed in herds participating in a RA-based JD control program in Minnesota (Wells et al., 2008). However, the average within-herd prevalence of the herds participating in that study was slightly higher than in this study.

Table 4. Summary of risk assessment (RA) scores for different management areas expressed as median, interquartile range (IQR), and range

Management area ¹	First RA (2005–2007)		Second RA (2008–2009)	
	Median (IQR)	Range	Median (IQR)	Range
CA ²	61 (49; 74)	14–106	51 (39; 61)	6–96
PW ²	42 (29; 50)	4–71	32 (20; 42)	2–69
W	3 (1; 8)	0–25	3 (1; 7)	0–31
BH	7 (4; 11)	0–21	7 (3; 10)	0–20
C	4 (2; 6)	0–15	4 (2; 6)	0–16
Total RA score	121 (94; 140)	32–187	95 (74; 121)	1–192

¹CA = calving area, PW = preweaned, W = weaned, and BH = breeding-age heifer, C = cow husbandry practices.

² $P < 0.0001$ for the difference between RA scores of the second and first RA.

Table 5. Distribution (n, % in parentheses) of apparent within-herd prevalence based upon a *Mycobacterium avium* ssp. *paratuberculosis* (MAP) antibody milk ELISA in 2005–2007 and 2008–2009

Apparent prevalence 2005–2007	Apparent prevalence 2008–2009			
	0%	1–5%	6–10%	≥11%
0%	114 (77)	30 (20)	4 (3)	0 (0)
1–5%	28 (51)	24 (44)	3 (3)	1 (2)
6–10%	2 (13)	9 (61)	2 (13)	2 (13)
≥11%	1 (13)	1 (13)	3 (37)	3 (37)

In the current study, the greatest decrease in within-herd prevalence was observed in herds with very high MAP-antibody ELISA-positive prevalence at test 1. The apparent prevalence in first-lactation animals decreased from 1.2 ± 3.8 to $0.5 \pm 2.0\%$ ($P = 0.0259$) among all herds and from 8.3 ± 6.7 to $1.8 \pm 3.4\%$ ($P < 0.0001$) in only the MAP ELISA-positive herds of test 1 between the 2 tests. The prevalence among older cows remained constant ($P > 0.18$) in the population, but decreased significantly if herds had previously been test-positive ($P \leq 0.0033$).

Sixty-five herds (28.7%) changed their MAP-antibody milk ELISA herd status between the 2 tests in this study (Table 5). Of the 34 farms that changed from test-negative to test-positive, 29 farms (85%) reported no clinical JD cases in the 12 mo before the first RA. However, 5 of the 34 herds had clinical JD cases on the farm in the preceding year (one herd had 5 known clinical JD cases) and 32% of these 34 farms had known JD cases in the 10 yr before the first JD RA. In addition, most of these operations (85%) were known to have purchased cattle in the years before the first RA,

which is a known risk factor for the introduction of subclinically MAP-infected cattle into a herd (Doyle, 1956; Wells and Wagner, 2000). However, although the change of MAP-antibody milk ELISA herd status was, in most cases, due to only 1 test-positive cow (59%), some herds had 2 ($n = 8$), 3 ($n = 3$), 4 ($n = 2$), or even 7 ($n = 1$) test-positive cows at test 2.

The culling of test-positive cows after the first RA did not result in a greater average decrease in apparent prevalence on those farms ($P > 0.12$). On the contrary, test-positive farms at test 1 that had culled MAP-antibody milk ELISA-positive cows had a slightly higher MAP-antibody milk ELISA-positive prevalence at test 2 (median = 1.95) than those farms that did not cull cows (median = 0; $P = 0.0461$). In addition, herds that did not cull tended to be more likely to be test-negative at test 2 compared with those that culled MAP-antibody milk ELISA-positive cows ($P = 0.0652$). Whether this was due to a lower rate of implementation of recommended management practices after culling the test-positive cows in test-positive herds (Sorge et al., 2010a) is unclear at this point, because no differences

Table 6. Apparent Johne's disease (JD) prevalence at the herd level and by lactation as determined by herd testing in 2005–2007 and 2008–2009 using a *Mycobacterium avium* ssp. *paratuberculosis* (MAP) antibody milk ELISA in 226 Canadian dairy herds

Parameter	2005–2007	2008–2009
Apparent within-herd prevalence, ¹ %	5.4 ± 5.2	4.2 ± 3.6
Maximum apparent prevalence, %	34.6	18.2
Maximum number of test-positive cows within herd, n	13	9
Test-positive herds (≥1 test-positive cow), %	34.5	35.8
Test-positive herds (≥2 test-positive cows), %	20.4	20.4
Age of test-positive cows, mo (mean ± SD)	57.7 ± 21.7	58.7 ± 20.7
Number of cows tested with MAP-antibody ELISA, n	13,702	15,184
Cow-level apparent prevalence in population, %		
Lactation 1	1.0	0.6
Lactation 2	1.8	1.7
Lactation 3	1.8	1.8
Lactation ≥4	2.6	2.0
Apparent prevalence by lactation within herds, %		
Lactation 1 ²	1.2	0.5
Lactation 2	2.2	1.8
Lactation 3	2.5	2.2
Lactation ≥4	2.7	2.2

¹For herds with ≥1 MAP-antibody milk ELISA positive cow (Mean ± SD).

² $P = 0.0259$ for difference between first and second test.

in the changes in RA scores were observed between test-positive herds that culled and did not cull ($P > 0.17$). However, the latter might be simply due to low statistical power, because only 78 herds were classified as test-positive at test 1.

The prevalence of MAP-antibody milk ELISA-positive results among first-lactation cows in 2008–2009 was not associated with any of the RAS or changes in RAS, with the exception of the time the calf spent with its dam after birth (CA12, $r = -0.131$; $P = 0.0495$). The longer the time the calves spent with their dam, the lower the prevalence of MAP-antibody milk ELISA-positive cows was among the first-lactating cows. However, this correlation was not consistent. When the analysis only included herds that were test-positive in 2005–2007, the relationship was not significant ($P > 0.10$). This might indicate that test-negative herds were more likely to leave newborn calves with their dam.

Few changes in RAS for individual RA questions could be correlated with the change in the MAP-antibody milk ELISA-positive prevalence of first-lactation animals. The only exceptions occurred when only test-positive herds at test 1 were selected. When using the subset of these herds, the change in the prevalence of first-lactation cows was correlated with changes in feeding frequency of nonsaleable milk (PW3, $r = 0.239$; $P = 0.0476$) and hygiene in the weaned calf area (W4, $r = 0.206$; $P = 0.0868$). When less nonsaleable milk was fed and manure contamination of the feeding equipment for weaned heifers became less severe, the MAP-antibody milk ELISA prevalence decreased to a greater degree in a herd's first-lactation animals. Although no further statistical association was found between changes in RAS and changes in the prevalence of first-lactation animals, the observed decrease in prevalence in first-lactation animals could still indicate that suggested management changes were effective in reducing the exposure of young calves to MAP. Collins et al. (2010) reported a similar decrease in prevalence in first-lactation cows on most farms participating in an RA-based JD control program over a period of 6 yr, suggesting that the implementation of a management-based JD control program might indeed lead to decreased JD and MAP infection prevalence.

However, a limitation of this and other studies using MAP-antibody ELISA is that the true infection status of a herd or cows is unknown. In addition, like any other test for MAP infections, the MAP-antibody milk ELISA is imperfect and might lead to an overestimation of prevalence in herds with low true prevalence. Therefore, to determine the magnitude of MAP transmission through individual management practices, repeated MAP culture from cows and environment is necessary to identify not only the true MAP infection

prevalence of herds, but also the transmission patterns through selected management practices. Future studies will need to culture MAP to quantify the actual MAP transmission through the potential high-risk management practices identified herein. Because many farmers rely on ELISA results rather than expensive culture, this study focused on changes in MAP-antibody milk ELISA prevalence due to changed management procedures. Therefore, we emphasize that the results of this study should only be interpreted in accordance with the study objectives; that is, association of management changes with changes in MAP-antibody milk ELISA prevalence of herds.

CONCLUSIONS

The herds in this study were a self-selected sample of the dairy industry in Ontario and western Canada. Throughout the course of this study, only minor changes in the distribution of management practices were observed. Some of the changes made could be linked directly to compliance with recommendations made by herd veterinarians after the first RA was conducted, indicating that the RA accurately reflected management practices on these farms. On average, the herds decreased their apparent within-herd MAP-antibody milk ELISA prevalence over the study period. Although the majority of herds remained either test-positive or test-negative, almost one-third of herds changed their MAP-antibody milk ELISA herd status. This finding emphasized that a MAP-antibody ELISA herd test has to be current to accurately reflect the present test status of a herd.

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