High herd-level prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Western Canadian dairy farms, based on environmental sampling

R. Wolf,*¹ H. W. Barkema,* J. De Buck,* M. Slomp,† J. Flaig,† D. Hauptstein,‡ C. Pickel,* and K. Orsel*

*Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

†Alberta Milk, Edmonton, Alberta, Canada T6X 1H1

‡SaskMilk, Regina, Saskatchewan, Canada S4N 4Y1

ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes chronic progressive enteritis in ruminants. The pathogen is present in most countries with modern dairy production, causing substantial economic losses for the industry. The objectives of this study were to estimate dairy herd prevalence of MAP in the Western Canadian provinces of Alberta and Saskatchewan, and to determine whether herd size and housing system (tie-stall versus freestall or loose housing) affected the risk of a herd testing positive for MAP. Six environmental samples were collected on 360 Alberta farms (60% of registered producers) and on 166 Saskatchewan dairy farms (99%). In total, 47% of the sampled farms in Alberta and 53% of the sampled farms in Saskatchewan had at least one environmental sample that was MAP culture positive and were, therefore, defined as infected. Sensitivity of environmental sampling was estimated using 3 subsequent annual tests performed on 82 farms. Because laboratory protocols were continuously improved throughout the project, the sensitivity increased over time. Therefore, a mean of the sensitivity estimates weighted on sampling year was constructed; this resulted in sensitivities of 68 and 69% for Alberta and Saskatchewan, respectively. Implementing those estimates in an approximate Bayesian computation model resulted in a true herd prevalence of 68% (95% probability interval: 60–80%) for Alberta and 76% (95% probability interval: 70–85%) for Saskatchewan. Herds with >200 cows had 3.54 times higher odds of being environmental sample positive and had more positive samples than herds with <50 cows (neither province nor housing system affected those results). In conclusion, the majority of Alberta and Saskatchewan dairy farms were infected with MAP and larger herds were more often MAP positive than smaller herds.

Key words: prevalence, *Mycobacterium avium* ssp. *paratuberculosis*, latent class analysis, environmental sampling

INTRODUCTION

Johne's disease is a chronic progressive enteritis, caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP), affecting most ruminant species (Fecteau and Whitlock, 2010). The disease is present in most countries with a modern dairy industry and causes substantial losses through decreases in milk production and slaughter value, combined with an increased risk of being culled (McKenna et al., 2006; Barkema et al., 2010b). For Atlantic Canada, the actual financial losses were estimated to be \$2,472 for a 50-cow herd with 7% MAP-infected cattle (Chi et al., 2002). A second concern associated with MAP is its potential zoonotic nature. Although controversial, a link may exist between MAP infection in cattle and Crohn's disease in humans (Barkema et al., 2010a). Should this link ever be proven, lost consumer trust as well as a decreased milk price are expected (Groenendaal and Zagmutt, 2008).

The herd prevalence of MAP on Alberta dairy farms is uncertain, as estimates range from 20 to 59% (Sorensen et al., 2003; Scott et al., 2006). Only 1 study estimated a relatively low herd prevalence of 24% for Saskatchewan (VanLeeuwen et al., 2005). It is noteworthy that most estimates were based on testing a subset of cows in a herd using serum ELISA, which has low sensitivity and low specificity (Tiwari et al., 2006). To avoid overestimation of herd prevalence due to poor specificity, the authors defined only herds with at least 2 cows positive on serum ELISA as MAP infected. However, this approach only adjusts for a lack in specificity, but not for a lack in sensitivity; therefore, reported herd prevalence estimates are likely an underestimation of true prevalence (Barkema et al., 2010b).

Environmental sampling is another potential testing strategy used to detect herds with cows infected with

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¹Corresponding author: rwolf@ucalgary.ca

MAP. It is currently used in prevention and control programs in the United States and in some parts of Canada, including Alberta [Alberta Johne's Disease Initiative (**AJDI**)] and Atlantic Canada (Whitlock, 2010; AJDI, 2011; Mason, 2012; Wolf et al., 2014). Trained sampling personnel collect manure samples from the cows' environment and manure storage areas. The advantages are that it does not require sample collection from individual animals, and it relies on the almost perfect specificity of bacterial culture, which simplifies true prevalence estimation (Tiwari et al., 2006). The testing method is reliable and the sensitivity, compared with fecal testing of individual cows, is approximately 70% (Raizman et al., 2004; Lombard et al., 2006; Aly et al., 2009).

Herd size is positively associated with risk of MAP infection, with almost all large dairy herds in the United States being reported as infected (Wells and Wagner, 2000; USDA, 2008; Pillars et al., 2009). Possible reasons for this association are differences in management practices, such as an increased use of pooled colostrum or using group calving pens in large herds, both of which increase the risk of within-herd transmission of MAP (Stabel, 2008; Pithua et al., 2013), and also more purchased animals in large herds, which increases the risk of MAP introduction (Wells and Wagner, 2000). However, herd size distributions and management practices are area specific, which limits the generalizability of results. Knowing the effect of herd characteristics, such as herd size and housing system, on the risk of MAP infection, in combination with local prevalence estimates, will provide individual dairy farmers with valuable information regarding the actual risk of MAP infection on their farm. This information can be used to estimate the importance and cost-effectiveness of management practices implemented to reduce the within-herd transmission of MAP. Knowing the herd prevalence in a population can also be used to estimate the risk of MAP introduction into an uninfected herd through the purchase of animals. The objectives of the current study were to estimate herd prevalence of MAP infection in Alberta and Saskatchewan dairy herds, based on environmental sampling, and to determine whether housing type or current herd size influenced the risk of MAP infection for a herd.

MATERIALS AND METHODS

Herds

At the beginning of the study (December 2010), the Alberta dairy industry consisted of 597 dairy farms. During the 3 yr of the study, 50 farms ceased their dairy operations and 24 new farms started produc-

ing milk. The Alberta study population consisted of herds voluntarily participating in the AJDI. Herds were visited annually by their herd veterinarian to collect environmental samples, followed by a risk assessment and suggested management changes (Wolf et al., 2014). Farms could join or leave the project throughout the study. Furthermore, 166 of 167 Saskatchewan dairy farms were visited from August 2012 to November 2013 by either their herd veterinarian (16 farms) or by a single employee of the producer organization SaskMilk (Regina, SK, Canada) who visited 150 farms (SaskMilk, 2012).

Herd size and lactating cow housing (tie-stall versus freestall or loose housing) of Alberta participants were assessed as part of the AJDI through questions in the annual risk assessment. For farms in Saskatchewan, information on housing was noted by the sample collectors and herd size (in increments of 50 cows) was estimated based on milk quota.

Sample Collection

Sample collectors received standardized training through AJDI workshops or one-on-one training sessions. Collectors received sampling kits containing 6 zip-lock bags for mixing subsamples, 6 sample containers (90 mL), and an instruction sheet. Duplicate samples were collected from 3 areas: (1) manure storage (e.g., lagoons, piles, or pits), (2) manure concentration (e.g., alleys and the end of scraper lines), and (3) cow concentration, including sick-cow pens (Berghaus et al., 2006). If manure was not accessible in manure storage areas, or if <2 cows were present in the sick-cow pens, collectors were instructed to collect additional samples from the remaining areas. Each sample consisted of at least 4 subsamples, which were thoroughly mixed inside the zip lock bags, and the fecal mix was subsequently transferred into containers. Samples were collected between Monday and Wednesday and shipped to the University of Calgary (Calgary, AB, Canada) using Express Mail. When samples were collected after Wednesday, collectors were instructed to keep samples refrigerated and ship them the following Monday.

Laboratory Analysis

Upon arrival at the University of Calgary, samples were stored at 4°C for a maximum of 7 d. Sample processing started every Monday using a standardized 3-d decontamination protocol, followed by 48 d of culture using a TREK ESP culture protocol (Trek Diagnostic Systems Inc., Independence, OH; McKenna et al., 2005). All culture products were analyzed with conventional insertion sequence 900 (**IS900**) PCR, with previ-

ously described primers (Vary et al., 1990). Over the 3 yr of the project, the purchase of new equipment, such as a gel reading device and a new thermocycler, likely increased the sensitivity of the laboratory procedures. The laboratory protocol was also modified with the increased use of sequence element F57 quantitative PCR (qPCR) for confirmation of inconclusive IS900 results (mainly double bands, faint bands, or bands very close to the target area) from January 2012 onward (Slana et al., 2008). Before that, laboratory personnel based their decision solely on IS900 results. Thereafter, the case definition was either positive on IS900 conventional PCR or positive on F57 qPCR (performed following inconclusive IS900 outcomes).

Statistical Analyses

Test sensitivity and prevalence were estimated using the Excel software (Microsoft Corp., Redmond, WA) add-in @RISK (Palisade Corp., Ithaca, NY), whereas data management and analysis of predictors for positive environmental sample results were done with Stata 11 software (StataCorp LP, College Station, TX).

Test Accuracy. Environmental sample results of the farms participating in the project for 3 yr were used to estimate the sensitivity of environmental sampling (Figure 1). It was assumed that culture of 6 environmental samples per farm would result in 1 false-positive farm out of 100 uninfected farms (specificity: 99%). For sensitivity estimation of environmental testing, the 3-yr test result was used as the gold standard. The assumption was made that the herd infection status did not change throughout the duration of the project, which is justified by the endemic nature of the disease, and that

the scientific literature apparently does not include any reports of successful eradication of the pathogen from a farm without complete depopulation. Notwithstanding, introduction of MAP infection could have occurred during the 3 yr. Herds that had at least 1 positive result in 3 yr were considered positive. Beta distributions were constructed with the following parameterization: α = herds positive during the specific year of testing and β = herds negative during the specific year of testing, but positive during at least 1 of the other years. As it can be assumed that not all infected herds were detected in 3 test events, the default analysis assumed that 20% of the 3-times-negative herds would be infected with MAP. The rationale was derived from 2 studies estimating the sensitivity of repeated environmental sampling compared with pooled fecal culture (Khol et al., 2009; Lavers et al., 2013). Khol et al. (2009) collected samples over a 6-mo interval on 26 farms, whereas Lavers et al. (2013) used a 3-mo interval for 32 herds. Means of the 3 sensitivity estimates weighted by the number of herds visited in 2011, 2012, and 2013 were constructed for both provinces.

Two additional sensitivity estimates were calculated to account for uncertainty around the parameter. The first sensitivity estimate (high sensitivity) assumed that none of the 3-times-negative herds would be infected with MAP (instead of 20% infected herds among the 3-times-negative herds in the default analysis). The second sensitivity estimate (low sensitivity) assumed that 50% of the 3-times-negative herds would be infected. The rationale for this sensitivity analysis was based on a 5-yr longitudinal study using pooled fecal culture that concluded only 50% of infected herds could be detected with 3 test events (Kalis et al., 2004).

Figure 1. Flow diagram illustrating the number of herds in various stages of the Alberta Johne's Disease Initiative and the sample used for estimating herd prevalence of *Mycobacterium avium* ssp. *paratuberculosis*.

Prevalence Estimation. The apparent prevalence was calculated, including the most recent sample set collected on each participating dairy farm. A sample set was deemed positive if at least 1 environmental sample was positive. Apparent prevalence and accuracy estimates were used to calculate the true MAP herd prevalence in the 2 provinces. The true herd prevalence represented the proportion of herds with at least 1 infected cow and was estimated separately for each of the 3 sensitivity estimates (default, high sensitivity, and low sensitivity). Analysis was performed using approximate Bayesian computation (ABC). Compared with traditional Bayesian analysis methods, ABC estimates posterior distributions without specifying a likelihood function and, therefore, can solve more complex analytical tasks (Turner and Van Zandt, 2012). The most important feature in the present model was incorporation of finite population sizes, which enables prevalence estimation for sample sizes, which are close to the population sizes. The advantage of the method is that it results in lower posterior uncertainty (smaller probability intervals) compared with Bayesian analysis using binomial models (Branscum et al., 2004; Su et al., 2004). In Equation 1 (Appendix), the ABC rejection model estimated the number of positive farms using a uniform distribution between 0 and the population size (M). If this number equaled the number of positive farms estimated from a hypergeometric distribution using the sample size (n), number of positive farms (x), and M, the iteration was accepted; otherwise, it was rejected (Appendix Equations 2 and 3). The last step incorporated test accuracy and translated the outcome into a proportion (Appendix).

Herd Size, Housing, and Province. The association between the dichotomous environmental sample results (0 positive samples vs. ≥ 1 positives) and herd size (<50, 50–100, 100–150, 150–200, or >200), housing type (tie-stall: yes/no), province (Alberta or Saskatchewan), and sampling year (2011, 2012, or 2013) was analyzed using chi-squared tests on contingency tables. Because the majority of tie-stall herds had <100 cows, the association between environmental sampling results and housing would be biased by herd size. Therefore, to determine whether the herd MAP prevalence was different among housing types, a separate analysis was performed including only herds with <100 cows. Thereafter, associations between categorized environmental sample results (0 positive samples, 1–3 positives, or 4–6 positives) and all predictors were analyzed using ordinal logistic regression. Parameters were included using forward selection. Criteria for inclusion in the final model were $P < 0.05$ or evidence for confounding (>20% change of any other coefficient if tested coefficient was removed). The validity of the propor-

tional odds model was tested using a Brant test. In case of validity, the same set of predictors can be used to describe an association for 2 different cut points in the outcome. For the present study, this would be the predictor specific odds ratio for a herd having no positive environmental sample versus ≥ 1 positive environmental sample, and the odds ratio for a herd having ≤ 3 positive environmental samples versus >3 positive environmental samples (Brant, 1990).

RESULTS

Herd Characteristics

Alberta. Three hundred sixty (60%) Alberta dairy farms participated in the AJDI, including 122 located south of the city of Calgary, 194 between Calgary and Edmonton, and 44 north of the city of Edmonton (participation rates of 74, 55, and 59%, respectively). Overall, 114 (31%) farms had <100 cows and 21 (6%) housed their cows in tie-stalls. Average herd size was 140 cows and 82% participated in an organized milk recording system (CanWest DHI, Guelph, ON, Canada). Only 17 farms (5%) submitted samples in 2011; 123 farms (34%) submitted their most recent sample set in 2012 and 220 (61%) in 2013 (Table 1).

Saskatchewan. In total, 166 (99%) Saskatchewan dairy farms were sampled, of which 66 (40%) were visited in 2012 and 100 (60%) in 2013. Of these farms, 78 (47%) had <100 cows, 103 (62%) participated in CanWest DHI milk recording, and 24 (14%) housed their cows in tie-stalls (Table 1).

Test Accuracy

On 82 Alberta farms, environmental samples were collected in 3 consecutive years. Of these farms, 59 (72%) had at least 1 positive environmental sample result during at least 1 sampling event. Furthermore, 19 (32%) of those 59 farms were positive in yr 1, 39 (66%) positive in yr 2, and 48 (80%) positive in yr 3 (Table 2). Assuming 20% false negatives in the 23 repeatedly test-negative farms, the sensitivity of environmental sampling weighted by the number of farms visited in each year was estimated at 68% [95% probability interval (PI): 60–76%] for Alberta. For Saskatchewan, where a higher proportion of farms was visited in 2013, sensitivity was 69% (95% PI: 60–77%). Assuming 100% sensitivity of 3 test events resulted in an annual sensitivity of 73 and 74% (high sensitivity), whereas assuming 50% of the 3-times-negative farms as infected resulted in a sensitivity of 62 and 63% (low sensitivity), respectively, for Alberta and Saskatchewan (Table 3).

Table 1. Associations between herd characteristics and the most recent environmental sample results estimated on 360 Alberta and 166 Saskatchewan (Canada) dairy farms

Item	Alberta ^{1,2}				Saskatchewan ^{1,2}			
	0 pos. [no. (%)]	1–3 pos. [no. (%)]	4–6 pos. [no. (%)]	Total no.	0 pos. [no. (%)]	1–3 pos. [no. (%)]	4–6 pos. [no. (%)]	Total no.
Total	191 (53)	92 (26)	77 (21)	360	78 (47)	50 (30)	38 (23)	166
Region								
South of Calgary	72 (59)	35 (29)	15 (12)	122	Not applicable			
Calgary - Edmonton	94 (48)	50 (26)	50 (26)	194				
North of Edmonton	25 (57)	7 (16)	12 (27)	44				
Number of cows								
<50	6 (67)	1 (11)	2 (22)	9	9 (50)	7 (39)	2 (11)	18
50–99	69 (66)	24 (23)	12 (11)	105	34 (57)	14 (23)	12 (20)	60
100–149	73 (53)	39 (28)	26 (18)	138	21 (57)	10 (27)	6 (16)	37
150–200	25 (49)	11 (22)	15 (29)	51	10 (40)	10 (40)	5 (20)	25
>200	18 (32)	17 (30)	22 (39)	57	4 (15)	9 (35)	13 (50)	26
Lactating cow housing								
Freestall/loose housing	175 (52)	88 (26)	76 (22)	339	63 (44)	44 (31)	35 (25)	142
Tie-stall	16 (76)	4 (19)	1 (5)	21	15 (63)	6 (25)	3 (13)	24
Year of sample collection								
2011	15 (88)	1 (6)	1 (6)	17	—	—	—	—
2012	74 (60)	21 (17)	28 (23)	123	32 (49)	18 (27)	16 (24)	66
2013	102 (46)	70 (32)	48 (22)	220	46 (46)	32 (32)	22 (22)	100

¹Number of positive (pos.) environmental samples during the last testing event.²Percentages represent row percentages.**Table 2.** Environmental sample results (testing positive or negative) for herds participating in the Alberta Johne's Disease Initiative over 3 consecutive years (n = 82)

Year 1 (represents 2010/2011)	Year 2 (represents 2012)	Year 3 (represents 2013)	Farms [no. (%)]
+	+	+	12 (15)
+	—	+	3 (4)
—	+	+	17 (21)
—	—	+	16 (20)
+	+	—	3 (4)
+	—	—	1 (1)
—	+	—	7 (9)
—	—	—	23 (28)

Table 3. Sensitivity and specificity estimates of environmental sampling, and herd prevalences for *Mycobacterium avium* ssp. *paratuberculosis* in Alberta and Saskatchewan (Canada) dairy farms [median (95% probability interval)]

Item	Default ¹	High sensitivity ²	Low sensitivity ³
Sensitivity, Alberta ⁴	0.68 (0.60–0.76)	0.73 (0.66–0.80)	0.62 (0.54–0.68)
Sensitivity, Saskatchewan ⁴	0.69 (0.60–0.77)	0.74 (0.66–0.82)	0.63 (0.55–0.70)
Specificity	0.99 (0.96–1.00)	Default	Default
Prevalence, Alberta	0.68 (0.60–0.80)	0.64 (0.56–0.72)	0.76 (0.66–0.90)
Prevalence, Saskatchewan	0.76 (0.70–0.85)	0.70 (0.65–0.78)	0.84 (0.76–0.94)

¹The default analysis assumed that 20% of the repeatedly test-negative herds were infected.²High sensitivity assumed that all positive farms would be detected with 3 environmental sampling events; hence, assumed 3 consecutive testings as the gold standard.³Low sensitivity assumed that 50% of the repeatedly test-negative herds were infected.⁴Sensitivity estimates were based on consecutive test results from 82 Alberta dairy farms. Estimates represent weighted averages of the annual sensitivities and varied between the provinces due to different sampling weights.

Prevalence Estimation

In total, 47% of the farms in Alberta and 53% of the farms in Saskatchewan had at least 1 environmental sample that was MAP culture positive and were, therefore, defined as infected (Table 1). The default analysis resulted in a herd prevalence of 68% (95% PI: 60–80%) for Alberta and 76% (95% PI: 70–85%) for Saskatchewan (Table 3). The high sensitivity and the low sensitivity analysis resulted in a change of the prevalence estimates of –8 and +8% respectively, compared with the default analysis (Table 3).

Herd Size, Housing, and Province

Whereas 74 (38%) of 192 herds with <100 cows tested positive, 61 (73%) of 83 herds with >200 cows tested positive, resulting in a significant effect of larger herds ($P < 0.001$; Tables 1 and 4). Fourteen (31%) of 45 tie-stall herds, and 243 (51%) of 481 freestall or loose housing herds tested positive ($P = 0.08$). In herds with <100 cows, 12 (35%) of 34 tie-stall herds and 62 (39%) of 158 freestall or loose housing herds tested positive. The 2 proportions were not significantly different ($P = 0.67$). The proportion of positive herds increased from 2011 to 2012 to 2013 (12, 44, and 54% respectively; $P = 0.001$). No evidence existed for an association between province and testing MAP positive ($P = 0.20$).

In the ordinal logistic regression model, herd size ($P < 0.001$) and year of sample collection ($P = 0.01$) were associated with the number of positive environmental samples collected on a farm (Table 4). Herds with >200 cows had 3.54 times higher odds to have at least 1 positive environmental sample and had a higher number of positive samples than herds with <50 cows. Herds

tested in 2012/2013 were more likely to test positive and had more positive environmental samples than herds tested in 2011 (Table 4). Province and housing in tie-stalls were not included in the final model. The Brant test for the final model was not significant ($P = 0.27$). Therefore, no evidence existed for a violation of the proportional odds assumption.

DISCUSSION

In the current study, 68 and 76% of Alberta and Saskatchewan dairy farms, respectively, were estimated to be infected with MAP, whereas large herds were more often MAP culture positive than smaller herds. To the authors' knowledge, this MAP prevalence study had by far the highest participation rate of any similar study, with >60% of the population participating in Alberta and 99% participating in Saskatchewan.

Herd-level MAP prevalence estimates were not different between provinces, but seemed to be higher than in other reports (Sorensen et al., 2003; VanLeeuwen et al., 2005; Scott et al., 2006). In one study, the proportion of herds with at least 1 ELISA-positive cow was very close to the prevalence reported in the current study (Scott et al., 2006). Therefore, we suspect that using a cutoff of 2 ELISA-positive cows most likely resulted in an underestimation of true prevalence. The substantially lower prevalence estimates by 2 other studies cannot be explained; we suspect that they either were due to a surprisingly large increase in prevalence over the last 10 yr, or more likely, an underestimation of the herd prevalence in those studies through systematic error (Sorensen et al., 2003; VanLeeuwen et al., 2005). This new knowledge on prevalence of MAP in Western Canada has important implications for MAP control. A recent

Table 4. Final ordered logistic regression model on the association between the number of positive environmental samples (in 3 categories: 0, 1–3, and 4–6 positive samples) and herd characteristics estimated on 360 Alberta and 166 Saskatchewan (Canada) dairy farms

Parameter	Coefficient	P-value	Odds ratio	95% CI
Herd size		<0.001 ¹		
<50 cows	Reference			
50–100 cows	–0.26	0.525	0.77	0.35–1.71 ²
100–150 cows	0.09	0.814	1.09	0.50–2.40 ²
150–200 cows	0.49	0.260	1.62	0.70–3.78 ²
>200 cows	1.27	0.003	3.54	1.54–8.17 ²
Year of sample collection		0.011 ¹		
2011	Reference			
2012	1.98	0.011	7.26	1.58–33.32 ²
2013	2.20	0.004	9.01	1.99–40.85 ²
Intercept				
0 vs. ≥1 positives	2.33	—	—	0.67–3.98 ³
≤3 vs. >3 positives	3.65	—	—	1.98–5.32 ³

¹Wald P-value for the parameter.

²Confidence interval around the odds ratio.

³Confidence interval around the coefficient.

study estimated the economic benefit of participation in the AJDI and concluded that participation was not only cost-effective for the average producer, but also for herds with low within-herd prevalence (Wolf et al., 2014). Because almost three-quarters of the herds are infected, most herds can expect a positive net benefit through participation in a management-based control program such as the AJDI.

Estimation of the true herd prevalence using environmental sampling was recently done in the United States and in Southern Chile (Kruze et al., 2013; Lombard et al., 2013). The estimated true herd prevalence of MAP seemed to be higher in the United States and lower in Southern Chile than in Western Canada.

Especially in the case of MAP testing, with limited knowledge on the accuracy of the tests used, true prevalence estimates depend on the assumed accuracy, even more so if only a single test is used (Branscum et al., 2004). Because culture protocols differ among institutions, local information (if available) was emphasized to estimate the accuracy for the protocol using repeated testing on 82 herds. Three years of repeated testing was used as a first step to estimate the sensitivity of an environmental sampling event. Subsequently, it was assumed that 20% of the 3-times-negative farms would be MAP infected (Khol et al., 2009; Lavers et al., 2013). The resulting sensitivity estimates of 68 and 69% differed slightly between the 2 provinces, due to different sampling weights. The estimates are the basis for the default analysis, which can be interpreted as the most likely true herd-level MAP prevalence in these 2 provinces. It should be noted that sensitivity estimates from this report were very similar to estimates reported in other test-evaluation studies (Raizman et al., 2004; Lombard et al., 2006; Khol et al., 2009; Lavers et al., 2013). Hence, the use of sensitivity estimates from the literature would have resulted in similar prevalence outcomes. The first sensitivity analysis (high sensitivity) assumed no false negatives among the 3-times-negative farms; therefore, the estimates can be interpreted as the lowest possible prevalence in the 2 provinces. The second sensitivity analysis (low sensitivity) described the worst-case scenario, assuming 50% of the 3-times-negative herds as infected.

The specificity of 6 environmental samples was assumed to be 99%. Other studies used 99.9 or even 100% as specificity priors (Kruze et al., 2013; Lombard et al., 2013). The first reason for the more conservative estimate was the fact that not 1 but 6 samples from a negative herd must be correctly deemed negative to assign the correct herd infection status. Herd specificity decreases with an increasing number of samples (Dohoo et al., 2003). The second reason was the theoretical possibility that an environmental sample could be con-

taminated with MAP bacteria by a source other than dairy cattle. As MAP is an intracellular pathogen that does not reproduce in the environment, possible sources for contamination would be beef cattle and other domestic ruminants such as goats, sheep, or farmed elk. The probability for that is relatively low, as most farms only have dairy cattle (M. Slomp and D. Hauptstein; personal observation) dairy cattle are generally housed separately from beef cattle and other ruminants, and their manure is stored separately. However, it is still a possibility that a very small number of samples was contaminated through other sources, which also justifies the use of a more conservative specificity prior. Although uncertainty exists in the specificity of environmental sampling, it should be kept in mind that variation of this parameter results in only minor changes in true prevalence estimates, as most herds are infected with MAP and the magnitude of uncertainty around specificity estimates is rather small (a conservative range would be between 96 and 99.9%) (Lombard et al., 2013).

Analyzing results of the 82 herds tested 3 times revealed that the proportion of positive farms increased over time. Assuming that MAP is an endemic disease with slow prevalence changes, the number of farms getting newly infected within 3 yr should be rather low, which provided evidence for a change in accuracy rather than a change in prevalence. As the laboratory at the University of Calgary was certified to perform MAP cultures throughout the project and the USDA certification does not allow any false positives, this change in accuracy was most likely due to increased sensitivity, which was attributed to ongoing protocol improvements and purchase of new equipment in the laboratory. Before 2012, laboratory personnel based the case definition solely on conventional IS900 PCR. In the absence of exact alignment of PCR bands with the target amplicon size, samples were conservatively called negative. Because the F57 sequence is highly specific for MAP, F57 qPCR was increasingly used to classify these inconclusive samples (which resulted in a higher number of positives). Further improvements were acquisition of new PCR machines, a new gel-reading device, and changes in PCR gel composition. These changes likely enabled detection of lower bacterial concentrations. As these protocol changes were implemented simultaneously, a single weighted average was created for sensitivity rather than treating changes in protocol as different tests. These protocol improvements resulted in a sensitivity increase over time from approximately 30% in the first year to approximately 77% in the third year. In a study done in the United States, reported sensitivity estimates were close to the first-year estimates (Smith et al., 2011); therefore, we

suspect that similar accuracy problems existed in other laboratories.

Large herds were more likely to be MAP positive, consistent with other studies (Wells and Wagner, 2000; Pillars et al., 2009). They were also more likely to have >3 positive environmental samples, which gives evidence for higher within-herd prevalence in large herds, as the number of positive environmental samples is associated with within-herd prevalence (Lavers et al., 2013). Why herd size is such an important risk factor for infection and higher within-herd prevalence is unknown. Perhaps differences in management contribute, as large farms are more likely to have more cows calving concurrently; thereby, they would be more likely to have more than 1 cow in the calving pen and to pool colostrum from several cows. Conversely, those farms would also be more likely to control these risk factors, as control expenses (e.g., colostrum pasteurizers) can be shared among more cows. A second explanation might be that larger herds probably purchase more replacement animals (often from multiple sources), which increases the risk of pathogen introduction. The National Animal Health Monitoring System (NAHMS) Dairy 2002 study suggests that large herds are managed differently compared with small herds (USDA, 2002), but further research is needed to identify risk factors for MAP infection in large herds. Housing system did not significantly predict the outcome if herd size was included in the final model, because almost all tie-stall farms had <100 cows. Therefore, their lower tendency of having culture-positive environmental samples can be explained by smaller herd sizes.

Although the participation rate in the AJDI was with 60% relatively high, not all Alberta dairy farms participated. Some farmers might be more likely to participate because they know that their herd is infected to receive advice for control strategies from their veterinarian. In contrast, other farmers might be less likely to participate because they do not want anybody to find out that their herds are MAP infected. The University of Calgary conducted a survey study among nonparticipants; preliminary results indicated that farmers have different reasons for nonparticipation, which are positively as well as negatively associated with the disease (C. Ritter and H. W. Barkema, Department of Production Animal Health, University of Calgary, Calgary, AB, Canada; manuscript in preparation). Also, average herd size as well as the proportion of farms participating in the milk recording program (CanWest DHI) was very similar between participants and the total Alberta dairy farm population (AAFC-DFC-DPAC-CDC, 2013). Nevertheless, despite the large sample size, a small effect of selection bias can-

not be excluded. Dependent on the direction of this selection bias, the herd prevalence in Alberta may be slightly over- or underestimated.

Environmental samples were collected by herd veterinarians and employees of the producer organization SaskMilk. Interobserver variability could induce bias. However, the impact of this bias was considered to be low, as all collectors received standardized training (workshops or personal information sessions) and sample collection followed a strict protocol outlined in an instruction sheet that accompanied every sampling kit. Results of a study in the United States supported the low impact of interobserver variability by estimating a high repeatability of environmental sampling between collectors (Aly et al., 2009).

Repeated testing of herds over 3 yr was used to estimate the sensitivity of environmental sampling. The assumption was made that these herds would not change their infection status within the duration of the project. It is not likely that any infected herds became uninfected within those 3 yr; no report of eradication of MAP from a herd without complete depopulation was found in the literature. A larger concern is that uninfected herds may have become infected during that timeframe. Unfortunately, no information on the number of newly MAP-infected herds in a population over a certain timeframe was available. However, the risk of new infections among these herds was likely lower than herds on average because of their participation in the AJDI and because they likely used stricter biosecurity measures and caution during animal purchase, which thereby reduced the risk of MAP introduction.

That only environmental samples were collected and no individual testing was conducted could be regarded as important limitations of this study. No doubt exists that repeated testing of all animals in participating herds would have resulted in higher accuracy. However, it was not feasible for a study that included >500 herds. An alternative approach with similar costs would have been collection of individual fecal samples from 30 cows, with subsequent processing in pools of 5 cows. Despite an increased workload for cow selection and sample collection, the laboratory costs would have been equal. However, a US simulation study estimated a lower sensitivity for pooled fecal culture of 30 cows compared with 6 environmental samples, likely due to the low number of tested cows within a herd (Tavornpanich et al., 2008). Nevertheless, that study reported high herd sensitivity for ELISA. The lower processing costs and, in the case of milk ELISA, the lower costs for sample collection would have allowed us to test more cows within each herd. Regardless, we decided to use environmental samples, as ELISA for detection of

a MAP antibody response likely have lower specificity than environmental samples, which complicates true prevalence estimation (Tiwari et al., 2006).

A limitation of the Saskatchewan data set was that exact herd sizes were not available. Consequently, herd size was approximated using the milk quota. Because none of the herds sell substantial amounts of milk directly to consumers, the potential for bias was low (D. Hauptstein, personal observation). It is noteworthy that the ordinal logistic regression used the farm-specific test result as the outcome and not the true infection status of the herd. Hence, some low-prevalence farms were misclassified as uninfected. If these misclassifications were herd-size dependent, differential misclassification would have resulted in biased estimates. The possibility exists that environmental sampling is more accurate in large herds than in small herds. In herds with the same prevalence, large herds will, because of the number of cows present, have higher odds that at least 1 cow sheds (compared with small herds), which could result in a higher sensitivity of environmental sampling in large herds. Conversely, the present protocol collected only 6 samples, with 4 subsamples within each herd. It might also be more likely on small farms, that the manure of an infected cow is collected with 1 of the 24 subsamples; that would reduce sensitivity in large herds. Considering these arguments and the missing evidence in the scientific literature, it cannot be concluded that the sensitivity of environmental samples was dependent on the herd size. Regardless, further research is needed to address these issues.

CONCLUSIONS

It was estimated that the majority of dairy farms in Alberta and Saskatchewan were infected with MAP. Furthermore, large herds were more likely to test positive than small herds.

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APPENDIX A

The ABC rejection model used to estimate true prevalence using finite population size was as follows:

M = population size;

n = sample size;

x = test-positive farms;

Se = test sensitivity;

Sp = test specificity;

NA = reject iteration;

Equation 1 = RiskIntUniform(0,M), uniform distribution between 0 and M (only integer values can occur);

Equation 2 = RiskHypergeo(n,Equation 1,M), where Hypergeo = hypergeometric distribution;

Equation 3 = IF(Equation 2 = x,Equation 1,NA());

Equation 4 = Equation 3/M;

Equation 5 = (Equation 4 + Sp – 1)/(Se + Sp – 1).