



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

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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

Received March 3, 2014.

Accepted July 7, 2014.

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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-

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