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Environmental sample characteristics and herd size associated with decreased herd-level prevalence of *Mycobacterium avium* subspecies *paratuberculosis*

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ABSTRACT

Environmental sampling is an effective method for estimating regional dairy herd-level prevalence of infection with *Mycobacterium avium* ssp. *paratuberculosis* (MAP). However, factors affecting prevalence estimates based on environmental samples are not known. The objective was to determine whether odds of environmental samples collected on farm changed culture status over 2 sampling times and if changes were specific for location and type of housing (freestall, tiestall, or loose housing), the sample collected (i.e., manure of lactating, dry, or sick cows; namely, cow group), and effects of herd size. In 2012–2013 [sampling 1 (S1)] and 2015–2017 [sampling 2 (S2)], 6 environmental samples were collected and cultured for MAP from all 167 (99%) and 160 (95%) farms, respectively, in the province of Saskatchewan, Canada. Only the 148 dairy farms sampled at both sampling periods were included in the analysis. A mixed effects logistic regression was used to determine whether differences between sampling periods were associated with herd size and sample characteristics (cow group contributing to environmental sample, type of housing, and location). In S1 and S2, 55 and 34%, respectively, of farms had at least 1 MAP-positive environmental sample. Correcting for sensitivity of environmental sampling, estimated true prevalence in S1 and S2 was 79 and 48%, respectively. Herds with >200 cows were more often MAP-positive than herds with <51 cows in both S1 and S2. The percentage of positive samples was lower in S2 compared with S1 for all sampled areas, cow groups contributing to samples, types of housing where samples were collected, and herd size categories. However, samples collected from dry cow areas had the largest decrease in MAP-positive samples in S2 compared with all other cow group samples. Herds that were MAP-negative in S1 with a herd size 51 to 100 or 101 to 150 were more likely to stay MAP-negative, whereas MAP-positive herds with >200 cows more frequently stayed MAP-positive. No difference was observed in the odds of a sample being MAP-positive among housing types or location of sample collection in both sample periods. Of all farms sampled, 104 (70%) did not change status from S1 to S2. In conclusion, when herd-level MAP prevalence decreased over the 3-yr interval, the change in prevalence differed among herd size categories and was larger in samples from dry cow areas. It was, however, not specific to other characteristics of environmental samples collected.
Key words: paratuberculosis, environmental sample, Johne's disease, herd size, Saskatchewan

INTRODUCTION

Johne's disease (**JD**), a chronic enteritis caused by the bacterium *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), has an adverse economic impact on the dairy industry worldwide due to decreased milk production, increased risk of culling, and decreased slaughter value (Tiwari et al., 2006; Lombard, 2011; Smith et al., 2017). No cure or effective vaccine for prevention of MAP infection is available; therefore, control programs are primarily based on decreasing risk of new infections within a dairy herd (Kalis et al., 2001; McKenna et al., 2006). Canadian JD control initiatives rely on detection of MAP-positive herds and subsequent risk assessments, resulting in changes to management practices to decrease new infections within a herd (Wolf et al., 2014b). Following detection of a positive herd, producers can opt for individual cow testing to remove infectious cattle; however, detection of these cattle is difficult due to the prolonged incubation period, unreliable diagnostics, and variability of immune and symptomatic responses (Marcé et al., 2010; Barkema et al., 2018). Due to high variability among diagnostic tests in test characteristics, prevalence estimates can vary sub-

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stantially; therefore, test sensitivities and specificities must be considered when estimating true prevalence (Barkema et al., 2018).

True cow-level prevalence estimates are difficult to estimate, as diagnostic tests rely on detecting latent and varying immune responses of cattle, or detection of the pathogen, which is intermittently shed in milk and feces. Infected cattle can be identified as negative if sampled at a time of no shedding or before immune responses develop, and this may result in low-prevalence herds being categorized as negative, despite MAP-infected cattle being present (Raizman et al., 2007; Donat et al., 2015). Fecal shedding can be intermittent and extent of shedding is highly variable (Mitchell et al., 2015), which has large consequences for transmission of MAP, as the primary route of infection is fecal oral. However, due to survivability of MAP in the environment for prolonged intervals (Whittington et al., 2004), environmental samples are a cost-effective and reliable method for detection of MAP-positive herds (Berghaus et al., 2006), and are currently used in control programs in the United States and Canada (Whitlock, 2010; Wolf et al., 2014b).

The most common sampling method protocol for environmental samples requires 6 samples to be collected from various locations on a dairy farm (Berghaus et al., 2006). The type of sample collected is important, as sample characteristics affect the likelihood of a MAP-positive result (Wolf et al., 2015). For example, environmental samples from the lactating cow area are more likely to be positive than those from sick/calving pens or dry cow pens; furthermore, samples collected from locations where manure from several cows accumulate (e.g., alley ways or lagoons) are more likely to be positive than bedded packs or manure piles (Wolf et al., 2015). These sample-type specific characteristics of environmental samples can be grouped based on cows contributing to the sample (cow group; i.e., lactating, dry, sick, and so on), type of pen that cows are housed in (housing type; i.e., freestall, tiestall, loose housing, and so on) and location collected (location type; i.e., alley, gutter, bedding pack, and so on). Additionally, larger herds are more likely to have MAP-positive samples and have higher within-herd prevalence than smaller herds (Wells and Wagner, 2000); however, there is no evidence that herd size affects sample-type-specific results (Wolf et al., 2015).

Accurate prevalence estimates are essential for control, surveillance, and monitoring effectiveness of a control program over time (Barkema et al., 2018). In long-term studies, herd MAP prevalence estimates decrease over time when control programs are in place (Collins et al., 2010; Sorge et al., 2011). Most programs used milk or serum antibody ELISA to estimate herd prevalence, although it is unknown how or if herd MAP prevalence estimates based on environmental samples are associated with characteristics of environmental samples (cow group, housing, and location) and interact to skew apparent changes in prevalence at various time points, or if they change in association with prevalence estimates. The prevalence of MAP-infected herds based on environmental samples in Saskatchewan, Canada, has been reported (Wolf et al., 2014a). However, stability of herd infection status and associations with herd size following implementation of a control program has not been documented. The objective was to determine if odds of environmental samples collected on-farm changing MAP culture status over the 2 sampling times were specific for location and type of housing (freestall, tiestall, or loose housing) the sample has been collected, whether it included manure of lactating, dry, or sick cows (cow group), and whether it was associated with herd size.

MATERIALS AND METHODS

Herds

Environmental samples were collected from all 167 and 160 dairy farms in the province of Saskatchewan in the first and second samplings, respectively, as part of the Saskatchewan JD surveillance program of the Saskatchewan Ministry of Agriculture (Regina, SK, Canada) and SaskMilk (Regina, SK, Canada), the Saskatchewan dairy producer marketing board. Only farms sampled at both sampling periods (n = 148) were included in the analysis. Farms were visited once by a SaskMilk field technician (Regina, SK, Canada) between August 2012 and October 2013 (sampling period 1; **S1**), and a second time between September 2015 and February 2017 (sampling period 2; **S2**) by the same technician. Mean interval between sampling periods was 3.5 yr (17 farms sampled after 2 yr, 59 farms after 3 yr, 51 farms after 4 yr, and 21 farms after 5 yr). Herd size was categorized into 5 categories: <51, 51–100, 101–150, 151–200, and >200 cows. Herd size information was collected at S1 and applied to farms at S2. Following the first environmental sample collection in S1, MAP-positive farms were offered an option to enroll in whole-herd testing (individual serum ELISA, individual fecal testing, or both), along with completion of a risk assessment by the herd veterinarian for improvement of management practices. Veterinarians were trained to identify MAP transmission-specific risk factors and make suggestions within dairy producers capabilities for improvement. Any suggested changes