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Risk factors associated with *Mycobacterium avium* subsp. paratuberculosis herd status in Québec dairy herds

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ABSTRACT

Paratuberculosis is a chronic and contagious enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Control of paratuberculosis is justified given the associated economic losses and the potential role of MAP in Crohn's disease in humans. Management practices that limit exposure of susceptible animals to MAP are more effective at reducing disease prevalence than testing and culling infected cows. The objective of this retrospective case-control study was to study the association between management practices and MAP status in dairy herds in Québec, Canada. A total of 26 case herds (MAP had been isolated from at least 1 environmental sample in each herd) and 91 control herds (no clinical cases of paratuberculosis and negative on 2 consecutive yearly environmental samplings) were selected among herds enrolled in the Québec Voluntary Paratuberculosis Control Program. A risk assessment questionnaire, completed at enrolment, was available for the selected herds. Culture of MAP was achieved using liquid media and the BACTEC 960 detection system. Multivariable logistic regression was used to evaluate the association between selected risk factors and MAP herd status. Herd size (OR = 1.17; 95% CI: 1.02-1.33) and proportion of cows purchased per year in the last 5 years (OR = 5.44; 95% CI: 1.23-23.98) were significantly associated with a positive MAP herd status.

The management risk factors identified in the present study are in accord with previous studies. Management practices aiming to prevent the introduction of new animals into the herd and to reduce the contact of newborn calves with adult animals or their feces are key elements to minimize MAP introduction and transmission into a herd. These elements should be prioritized in control programs.

1. Introduction

Paratuberculosis is a chronic and contagious enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Economic losses associated with paratuberculosis are related to lower milk production (Hendrick et al., 2005; Lombard et al., 2005), increased culling rate (Tiwari et al., 2005), and decreased carcass weight at slaughter (Kudahl and Nielsen, 2009). The pathogen MAP is often found in patients with Crohn's disease, a human chronic inflammatory bowel disease, but a causal relationship has not been confirmed (Waddell et al., 2015).

Cattle usually become infected at a young age, primarily by the fecal-oral route (Manning and Collins, 2010). After a long incubation period (up to several years), infected cows may start shedding MAP without showing clinical signs, thus perpetuating MAP infections on the farm (Benedictus et al., 2008). Management procedures that limit exposure of susceptible animals to MAP are more effective at reducing disease prevalence than simply testing and culling MAP infected cows (Garry, 2011). Thus, reducing the risk of transmission to susceptible young stock is of primary importance (Doré et al., 2012).

The possibility of direct or indirect contact between calves and adult cows or adult manure has been associated with paratuberculosis in previous studies. However, the definition of calf exposure to adult manure varies among these studies. For example, exposure to adults other than the dam at birth was associated with cows testing positive to MAP (Pillars et al., 2011), housing < 6 month old calves with adults

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(Dieguez et al., 2008), and exposure of 0–6-week-old calves to the feces of adults (Obasanjo et al., 1997) were associated with positive MAP herd status. Hygiene of the neonatal environment has been found to be an important risk factor. For example, contamination of the udders of periparturient cows with manure (Ansari-Lari et al., 2009) and the extent of manure buildup in calving area (Berghaus et al., 2005) have been reported to be associated with positive MAP herd status. Also, herd size (Wells and Wagner, 2000; Ridge et al., 2010; Bolton et al., 2011; Vilar et al., 2015) and introduction of new animals to the herd (Pillars et al., 2009; Correia-Gomes et al., 2010; Kunzler et al., 2014; Pieper et al., 2015) are frequently found to be risk factors.

Among the studies evaluating the association between risk factors and MAP herd status, serum ELISA and milk ELISA are the most common diagnostic tests used (Nielsen and Toft, 2011; Sorge et al., 2012; Pieper et al., 2015; Vilar et al., 2015). One recent study in Alberta, Canada (Wolf et al., 2016) determined MAP herd status using environmental fecal sampling. Bacteriologic culture of environmental samples is considered the most cost-effective strategy to determine herd status (Sweeney et al., 2012). It has been suggested that protocols to monitor MAP prevalence that include environmental sampling may be of great benefit to the global effort in the control and prevention of paratuberculosis (Barkema et al., 2010).

In Québec dairy herds, risk factors associated with MAP herd status might be different from those already reported in the literature. The distinctive characteristics of the dairy operations in this province − housing (92% of the herds are housed in tie-stall barns) (Groupe AGÉCO, 2013), herd size (average of 57 cows per farm) (Groupe AGÉCO, 2013), and the humid continental climate with long, cold winters − could affect the epidemiology of MAP on farms in this area.

The objective of this retrospective case-control study was to identify the association between management practices and MAP herd status (determined using bacteriologic culture of environmental samples) of dairy herds in Québec, Canada.

2. Material and methods

2.1. Study design

A retrospective case-control study was conducted to identify management practices associated with positive MAP herd status.

2.2. Source population, study sample and case definition

The unit of interest was the herd. The source population was composed of dairy herds enrolled in the Québec Voluntary Paratuberculosis Prevention and Control Program (QVPPCP) in 2012. This program was initiated in 2007. Upon enrollment, producers completed a general survey which included a section on herd characteristics and a section on risk factors. Fifty-nine potential risk factors were studied (Risk Assessment Questionnaire − RAQ, included in the supplementary material). No sampling was performed the first year of enrollment. Starting after the first year, yearly environmental samples were collected and cultured to detect the presence of MAP. The sampling frame included 330 herds that met the following inclusion criteria: enrolled for a minimum of 2 years in the QVPPCP, completion of a risk assessment questionnaire at enrollment, and raises replacement heifers on-site.

To complete the study sample, case herds were initially selected among the 330 herds of the sampling frame. A case herd was defined as a herd from which MAP had been isolated from at least 1 environmental sample. All available case herds were included ($n = 26$). The criteria to define a control herd were: no clinical cases of paratuberculosis and culture negative on 2 consecutive yearly environmental samplings. All herds meeting the criteria were included as controls ($n = 92$). Selection of cases and controls resulted in a ratio of approximately 3 controls per case. In the case of the present study, (26 case herds), if a predictor of interest has a 20% exposure in the control group, a ratio of 3 controls per case would have a 70% power to detect an odds ratio (OR) of 3 or higher with 95% confidence (Lewallen and Courtright, 1998).

2.3. Sample collection

Environmental samples were collected during the second year of enrollment of each herd in the QVPPCP. Sample collection and laboratory analysis were completed between 2009 and 2011. The following 3 sites were sampled in duplicate by the attending veterinarian for a total of 6 composite environmental samples per farm: 1) areas where feces from adult cows accumulate, 2) areas of manure storage, and 3) areas other than site 1 where feces accumulate (e.g.: calving area). Each composite sample consisted of about 20 g of manure or feces collected from 4 different surfaces within each site. Samples were stored in a plastic container, refrigerated at 4 °C, and shipped to the laboratory within 48 h after collection.

2.4. Laboratory testing

Bacteriologic culture was performed at the provincial laboratory (Laboratoire d'épidémiosurveillance animale du Québec, LÉAQ – Saint-Hyacinthe, Québec, Canada), which is an accredited laboratory for MAP culture by the United States Department of Agriculture. After arrival, samples were stored at −70 °C until the analyses were performed. Samples were processed as described elsewhere (Arango-Sabogal et al., 2016). Isolation of MAP was achieved using the MGIT Para TB culture media and the BACTEC 960 system (Becton, Dickinson and Company, Sparks, MD, USA). For BACTEC 12B, sensitivity to detect shedders was estimated between 26% and 89% (Eamens et al., 2000). Analytic sensitivity of the BACTEC 960 MGIT system has been reported to be 101 CFU (Shin et al., 2007). Sensitivity of environmental sampling for dairy herds has been reported to be between 32% and 71% with a specificity of 100% (Lavers et al., 2013; Arango-Sabogal et al., 2016).

2.5. Exposure measure

Exposure was measured using 59 questions related to MAP introduction and transmission (i.e. RAQ). Questionnaires were stored in a database (Access® 1997; Microsoft Corporation, Redmond, Washington, USA) and analyzed using Stata® Statistical Software (Release 14. College Station, TX: StataCorp LP).

2.6. Statistical analysis

2.6.1. Descriptive analysis

The unit of analysis was the herd. A descriptive analysis was initially performed to explore all variables. Variables were excluded if more than 10% of data was missing (Bennett, 2001). Categorical variables with multiple answers were dichotomized according to risk and distribution (details in supplementary material). Continuous variable herd size was scaled to evaluate the odds of being MAP positive for every 10 cow increase to facilitate the interpretation of the model estimates. Variables evaluating purchases (number of purchased adult cows in the last year and in the last 5 years, and number of animals (cows, heifers and bulls) purchased in the last 5 years) were transformed to 3 variables expressing the proportion of purchased animals per year. Distribution of data was graphically assessed with histograms and normal probability plots.

2.6.2. Univariable analysis

Univariable analyses were carried out to assess the association between the dependent variable (MAP herd status: case or control) and each independent variable using the Pearson's Chi-square and Fisher exact test for categorical variables, and Wilcoxon rank-sum test for continuous variables.