



Dam's infection progress and within-herd prevalence as predictors of *Mycobacterium avium* subsp. *paratuberculosis* ELISA response in Danish Holstein cattle

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ABSTRACT

Understanding the primary routes of transmission of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is pivotal to manage the pathogen in cattle herds. MAP is transmitted both vertically and horizontally, and both the dam's stage of infection and the prevalence in the population are therefore potentially important for MAP transmission control. The objective of this study was therefore to assess the dam's infection progress and the within-herd test-prevalence as predictors of MAP infection in Danish dairy cattle.

MAP specific antibody ELISA records from 95,025 dam-offspring pairs were combined with test-prevalence estimates from 939 Danish Holstein herds. The odds of testing ELISA-positive given the within-herd test-prevalence and the time-period a dam had had MAP specific antibodies were estimated for the offspring. Both dams and offspring were tested as adults, and parity-group was used to correct for the effect of age.

The results showed that both the within-herd test-prevalence and the dam's infection progress were significant predictors, while the dams that had tested positive when giving birth and up to 0.7 years after were more likely to have offspring that would test positive. The odds of testing positive were about 1.5 to 2.5 times higher for these offspring, compared to offspring of dams that never tested positive. Furthermore, offspring born in high (>5% ELISA-positive) and medium (2.5 to 5% ELISA-positive) prevalence herds had 9 and 3, respectively, times higher odds of testing positive, compared to animals born in a low prevalence herd. The variance heterogeneity reduced 81% through the included predictors.

The results of this study suggest that irrespective of the prevalence, offspring of dams with MAP specific antibodies should be considered as high-risk animals when managing the infection in cattle herds, but both the prevalence and the dam's infection status are important in MAP control.

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1. Introduction

Transmission of bovine *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is primarily via the faecal-oral route, where MAP faecally shed to the farm environment is taken up by susceptible individuals. The latter mostly include young stock, although adults are also susceptible to infection (Sweeney, 1996; 2011). MAP can also be shed directly in milk (Streeter et al., 1995; Sweeney et al., 1992a; Taylor et al., 1981), but transmission via milk and colostrum does not appear to be of upmost importance in control of MAP in

cattle (Nielsen et al., 2008; 2011). *In utero* transmission of MAP is also known to occur (Seitz et al., 1989), and the risk of *in utero* infection increases with the stage of infection of the dam (Sweeney et al., 1992b; Whittington and Windsor, 2009). Initially, many MAP infections are latent, while the infection is under control (Sweeney, 2011). Loss of control can be accompanied by occurrence of MAP specific antibodies, which are detectable by ELISA (Nielsen, 2014). The specific infection stage, where *in utero* transmission occurs has not been characterised in detail.

Theoretically, the risk of vertical transmission should depend primarily on the infection stage of the dam, and not the within-herd prevalence. However, perinatally the dam can transmit MAP vertically or horizontally given the proper conditions. A recent study suggested that in a contaminated environment, the exposure to a

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MAP excreting dam is not important (Eisenberg et al., 2015b). To the farmer, it is important to be able to predict the risk of transmission from the dam given the dam's infection stage in a given herd under the herd conditions prevailing, in practice often given by the MAP infection prevalence.

Our hypothesis was that a cow with MAP specific antibodies would have reached an infection stage where she would be more likely to transmit the infection to her offspring. However, the risk of infection after birth would be prevalence dependent, because herds with a high prevalence would also have a higher bacterial load. Therefore, the objective of this study was to assess the MAP ELISA status of the dam under different prevalence conditions as a predictor of the MAP ELISA status of a Danish Holstein cow.

2. Materials and methods

2.1. Herds and animals

The study was based on data from dairy herds enrolled in the Danish control program on paratuberculosis (Nielsen and Krogh, 2014). The data were obtained from the Danish Cattle Database, which is hosted by SEGES (Aarhus N, Denmark), the organization also administrating the paratuberculosis program. Enrolment implies that a herd is tested four times annually using a MAP specific antibody ELISA (see below), meaning that the individual cow is tested repeatedly. The program was launched in March 2006, but due to a change in test on the 15 October 2008, test-records obtained prior to this date were not used. Test-records, from the period 15 October 2008 until 28 June 2015, were used from all cattle fulfilling the following inclusion criteria: (a) minimum one test-record should be available for a dam at the time she gave birth to her offspring; (b) the offspring should have minimum one test-record, and since testing is done on milk samples, only lactating (and thereby adult) "offspring" were tested; (c) all test-records used in the study should have been recorded, while the herd was enrolled in the program; and (d) all cows recorded as Holsteins were included, to reduce a potential effect of breed (Jakobsen et al., 2000). All test-records from all other cattle present in a herd at the birth of an included animal were used to estimate the test-prevalence in the herd at the birth of the specific animal (see below).

2.2. Diagnosis

In the program, all diagnoses are based on screening of milk samples collected via the milk recording system. Only lactating animals are tested, and they are tested using a commercial MAP specific antibody ELISA, ID Screen® Paratuberculosis Indirect, according to the manufacturer's instructions (IDvet, Grabels, France). A sample was considered positive if the sample-to-positive ratio was >0.15. The sensitivity depends on the age of the tested animals and the median effective sensitivity in the population has been estimated to 60% (Kirkeby et al., 2015). The specificity at this cut-off has been estimated to 98.66% (Nielsen et al., 2013). Non-specific reactions frequently occur in the first days of lactations where the cow produces colostrum (Nielsen and Toft, 2012). Therefore, results from samples collected less than 5 days after calving were excluded. Cows with one positive sample were categorised as "positive". All results were reported to farmers as part of the control program, but it was not recorded if the results were used for risk management (Nielsen and Toft, 2011).

2.3. Predictors

The primary predictors according to the study hypothesis were the dam's MAP status and the herd prevalence. The first occurrence

of "test-positive" was used to estimate the time from testing positive to calving. To assess the effect of "infection progress", we used this time-parameter as a predictor, i.e., infection progress = (date dam tested positive – date of calving)/365. The time from positive to calving was almost normally distributed with long tails, and a linear effect was not expected. Therefore, both parameters were grouped into 8 approximately equally sized groups, based on the percentiles, while a ninth group included all those that never tested ELISA positive.

The prevalence of test-positive cows among the cows present in the herd at the time of testing of an up-coming dam was calculated at each dam's calving. This prevalence was used as a predictor for the given dam at the specific calving, but because the relationship was not linear and the distribution was very skewed, it was categorised into three groups (low: 0 to 2.5%; medium: >2.5 to 5%; and high: >5% test-prevalence). The overall and annual mean of the test-prevalence were also used to describe the overall and annual within-herd estimates for descriptive purposes.

The parity of both the dam and the offspring was used to create 3 parity groups, with both categories of animals split into parity 1, parity 2 and parities >2, to account for the effect of age (Eisenberg et al., 2015a; Nielsen and Toft, 2012).

2.4. Statistical analyses

Descriptive statistics were done to characterise the within-herd prevalence and to illustrate the apparent effect of dam status on the risk of testing positive as an offspring. Analytically, a random effects logistic regression model was created to compare the odds of testing positive as an offspring given the included predictors as fixed effects (see above) and herd and dam as random effects. Analysis was carried out in Stata 13 (StataCorp LP, College Station, TX).

All candidate variables were simultaneously offered to a full model, which was then reduced by backwards elimination. Variables with $P < 0.05$ were retained in the final model. Subsequently, their two way interactions were assessed and if significant were retained. In all models, herd and dam nested within the herd were included as random effect terms to capture the likely correlation in the odds of being ELISA-positive for offspring in the same herd, coming from the same dam.

Finally, variance components (VC) were estimated by the formula:

$$VC = \frac{\sigma_u^2}{\sigma_u^2 + \pi^2/3}$$

where σ_u^2 was the variance at the herd or the dam level and π is the mathematical constant for the ratio of a circle's circumference to its diameter, commonly approximated as 3.14159. To assess how much of the variation in the risk of turning ELISA-positive at the herd level was explained by the fitted covariates the VC from the final fitted model with covariates was compared to the VC from a model without any covariates (i.e., an intercept only model).

3. Results

A total of 95,025 dam-offspring pairs were identified in 939 of the 1,105 dairy herds available. Most (65,128 (82%)) of the dams had one daughter, while 12,401 (16%) had two daughters, 1,531 (2%) had three, 123 had four and 2 had five daughters. The herds' contribution of pairs ranged from 1 to 556 pairs per herd, with a median of 88 dam-daughter pairs.

The estimated test-prevalence in the population was 5.6% in 2008, and 3.8% in 2015, peaking at 7% in 2009. The within-herd prevalence distribution in the included herds over these years is shown in Table 1. The overall test-prevalence among all daughters

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