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Association of paratuberculosis sero-status with milk production and somatic cell counts across 5 lactations, using multilevel mixed models, in dairy cows

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ABSTRACT

The aim of this work was to investigate associations between individual cow *Mycobacterium avium* ssp. *paratuberculosis* (MAP) seropositivity, 305-d corrected milk production, and somatic cell count during 5 lactations lifespan in Portuguese dairy herds using multilevel mixed models. We used MAP serum ELISA (Idexx MAP Ac, Idexx Laboratories Inc., Westbrook, ME) results (n = 23,960) from all the 20,221 adult cows present in 329 farms and corresponding 47,586 lactation records from the National Dairy Improvement Association. Cows and farms were classified as positive or negative. Multilevel mixed models were used to investigate the association of cow MAP status with variation in milk production and somatic cell count. Cow MAP status, farm status, and lactation number were considered as independent variables. A quadratic function of lactation number was used to mimic the effect of lactation order on milk production. The models considered 3 levels: measurement occasion (level 1) within cow (level 2) and cow within farm (level 3). Four final models were produced, including all herds and cows, to address the effect of farm status (models 1 and 2) or the effect of cow status (models 3 and 4) on the outcome variables. Our results show that MAP status affects milk production. Losses are detectable from third lactation onward. During the first 5 lactations, positive cows accumulated an average loss of 1,284.8 kg of milk when compared with the negative cows. We also observed that somatic cell counts were higher in positive cows and a positive interaction occurs between cow status and lactation number, suggesting a positive association between MAP infection and increased somatic cell counts. Our results are in line with previous studies, suggesting a possible positive relation between cow milk production and susceptibility to MAP infection.

Key words: paratuberculosis, milk production, somatic cell count, multilevel mixed model

INTRODUCTION

Paratuberculosis, or Johne's disease, is a chronic granulomatous enteric disease affecting both ruminant and nonruminant animals caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**). No fully effective tools or strategies exist to prevent new infections or disease progression. Effects of MAP on animal welfare are relevant and the effect on dairy operations has been linked to impaired udder health, milk production (McAloon et al., 2016; Pritchard et al., 2016; Smith et al., 2016), and reproductive performance (Donat et al., 2014; Mato et al., 2015; Pritchard et al., 2017).

A strong association has been documented between MAP and Crohn's disease (Waddell et al., 2015), but important knowledge gaps to establish the causal path still exist (More et al., 2017). Genetic mimicry between protein epitopes of MAP and human proteins have been associated with autoimmune disorders (Davis, 2015; Sechi and Dow, 2015; Singh et al., 2016).

Diagnosis of MAP infection and measurement of infection effects are difficult because of the long incubation period, lack of diagnostic tests that accurately determine present and future status of the animal, disease dynamics at animal and farm level, and case definition of the positive animal. These factors contribute to the considerable number of studies available in the literature reporting varying effect estimates (McAloon et al., 2016) and prevalence (Nielsen and Toft, 2009). Control programs are mostly based on regular testing of adult animals' blood or milk samples with an ELISA test. Serum ELISA measures humoral response to the

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presence of MAP. During early stages of infection, a cellular immune response is produced, whereas antibody production develops in later stages of infection, which translates to different test accuracy in different stages of infection (Stabel et al., 2014; Laurin et al., 2015, 2017). Although serum ELISA specificity is considered to be greater than 97%, sensitivity can vary from 15 to 75% depending on the stage of the disease (Timms et al., 2011; Mitchell et al., 2015) and also on milk yield, lactation, herd prevalence, DIM, milk protein, and SCC (Eisenberg et al., 2015).

The disease is disseminated worldwide, and no countries have published enough information to claim freedom from MAP infection; true prevalence among cattle appears to be approximately 20%, and at least 3 to 5% in several countries, whereas between-herd prevalence was guesstimated to be $> 50\%$ (Nielsen et al., 2009). Correia-Gomes et al. (2010) reported a proportion of 45.9% positive farms and 2.3% apparent prevalence at cow level in northern Portugal. No official MAP control programs exist in Portugal; current control strategies are based on voluntary control programs. The national prevalence or production effects have not been assessed thus far. The purpose of the present study was to analyze the effect of individual cow's seropositivity to MAP on 305-d corrected milk production and SCC based on data from the first 5 lactations of each cow in Portuguese dairy herds, using multilevel mixed model (**MLM**) given the hierarchical nature of the data.

MATERIALS AND METHODS

Data Collection

This study combines a cross-sectional structure with a longitudinal component. First, all cows ≥30 mo of age, present at the farms, were blood sampled to determine their MAP serological status. In the longitudinal component the milk production and SCC from first up to fifth consecutive lactations of those same cows were, retrospectively, recorded and used to assess the associations between MAP seropositivity, milk production, and SCC. For some cows, additional blood ELISA results were available and were used when establishing MAP status.

Data set 1 had 23,960 MAP ELISA blood serum results from 20,221 cows from 329 dairy farms enrolled in a Johne's disease voluntary control program in Portugal, designated BOVICONTROL run by SEGALAB (Laboratório de Sanidade Animal e Segurança Alimentar S.A., Argivai, Portugal) animal health laboratories, which is a dairy farmer-sourced company operating in Portugal. All cows present at those farms were tested if they were ≥30 mo. The ELISA tests were performed by SEGALAB animal laboratory (http://www.segalab.pt/web/guest/home), which operates under an ISO 17025 quality system (http://www.ipac.pt/pesquisa/ficha_lae.asp?id=L0295). A commercial kit (Idexx MAP Ab, Idexx Laboratories Inc., Westbrook, ME) was used according to the manufacturer's instructions (https://www.idexx.com/en/livestock/livestock-tests/ruminant-tests/idexx-paratuberculosis-screening-ab-test/). Idexx MAP ab is a monophasic indirect ELISA with the wells coated with a protoplasmic extract of MAP; before the assay, the sera samples were incubated with an extract of *Mycobacterium phlei* to neutralize any possible cross-reactions with atypical mycobacteria. The individual test results were assigned as follows: sample-to-positive ratio ≤45% was negative, ≥55% was positive, and dubious or suspect for results falling in between. Data set 2 was extracted from the National Dairy Improvement Association (**NDIA**) database (https://www.bovinfor.pt/Bovinfor/bovinfor.php) and had 305-d corrected lactation records from cows present in data set 1, corresponding to 47,586 cows. Lactations were included whether they were at least 305 d, or corrected according to Vasconcelos et al. (2004) if they were 210 and less than 305 d; this data set holds records from August 11, 1997, to March 19, 2013. Data set 3 was obtained from the Portuguese Veterinary Authority (DGAV, Lisbon, Portugal) and included the birth date, farm of birth, and between-farm movements for each cow. Data set 4 was created by the authors and gathers and expands the data from the previous data sets in a Microsoft Access 2013 (Microsoft Corp., Redmond, WA) database. The official unique ear tag identification (**ID**) number was chosen as the primary key for each cow. The final database contained cow ID number, birth farm and birth date (from data set 3), ELISA results (from data set 1), calving date, lactation number, lactation-weighted arithmetic average SCC within the lactation, 305-d corrected milk yield, and farm in which the lactation occurred (data set 2). Calculated fields in each record included daily average 305-d corrected milk production (**D305MP**), which was calculated by dividing 305-d corrected milk yield by 305 d, and natural log of the lactation-weighted arithmetic average SCC (**lnSCC**). The D305MP was used instead of 305-d corrected milk yield to avoid mathematical constrains in the convergence of the estimates of high numbers. Reports for statistical analysis were generated with the queries to the database. From the 20,221 cows ELISA tested, only those registered in NDIA (15,196) were used. Cow lactation and farm observations are presented in Table 1. Exploratory and descriptive analysis were performed using Excel 2013 (Microsoft Corp. Redmond, WA) and IBM SPSS Statistics, version 23 (IBM Corp., Armonk, NY).