J. Dairy Sci. 100:1–8
https://doi.org/10.3168/jds.2016-12267

Within-herd prevalence of intramammary infection caused by *Mycoplasma bovis* and associations between cow udder health, milk yield, and composition

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ABSTRACT

Subclinical mastitis is one of the major health problems in dairy herds due to decreased milk production and reduced milk quality. The aim of this study was to examine the within-herd prevalence of subclinical intramammary infection caused by *Mycoplasma bovis* and to evaluate associations between *M. bovis* and cow daily milk yield, udder health, and milk composition. Individual cow composite milk samples (n = 522) were collected from all lactating dairy cows in 1 Estonian dairy farm in November 2014. Daily milk yield, days in milk, and parity were recorded. Collected milk samples were analyzed for somatic cell count, milk protein, fat, and urea content. The presence of *M. bovis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis* in the milk samples was confirmed by quantitative PCR analysis. The within-herd prevalence of *M. bovis* was 17.2% in the study herd. No association was observed between days in milk and parity to the presence of *M. bovis* in milk. According to linear regression analysis, the daily milk yield from cows positive for *M. bovis* was on average 3.0 kg lower compared with cows negative for *M. bovis*. In addition, the presence of *M. bovis* in milk samples was significantly associated with higher somatic cell count and lower fat and urea content compared with milk samples negative for *M. bovis*. In conclusion, subclinical *M. bovis* intramammary infection is associated with decreased milk yield and lower milk quality.

Key words: *Mycoplasma bovis* mastitis, dairy cow, prevalence, milk yield

INTRODUCTION

Mastitis, which can be caused by different udder pathogens, is one of the major concerns in dairy herds because it causes economic losses to the industry due to lower milk production and reduced milk quality (Ruegg, 2012; Hertl et al., 2014). In addition, milk fat and protein concentration have been shown to decrease due to lipolysis and proteolysis in mastitic milk (Larsen et al., 2010; Vidanarachchi et al., 2015; Zhang et al., 2015).

The biggest effect on dairy herd milk quality and production arises from contagious mastitis pathogens such as *Staphylococcus aureus*, as well as *Streptococcus agalactiae* (Reksen et al., 2007; Paradis et al., 2010; Sørensen et al., 2010). *Mycoplasma bovis*, a bacterium lacking a cell wall from genus *Mycoplasma*, mainly causes IMI (Nicholas and Ayling, 2003; Maunsell et al., 2011). *Mycoplasma bovis* is usually classified as a contagious mastitis pathogen (USDA APHIS, 2008; Royster and Wagner, 2015). Transmission between animals occurs mainly at the milking time (Ruegg, 2012). *Mycoplasma bovis* usually causes subclinical or mild clinical IMI, which can progress to chronic mastitis. Severe clinical mastitis outbreaks may also develop (Bushnell, 1984; Pothmann et al., 2015; Ruegg and Erskine, 2015). *Mycoplasma bovis* mastitis is not treatable with antibiotics; therefore, the control strategies of *M. bovis* IMI are to keep the herd uninfected, and to segregate and cull infected cows (Fox et al., 2005; Royster and Wagner, 2015; Nicholas et al., 2016).

Traditionally, *M. bovis* has been identified using culture-based methods, but due to low sensitivity and a long incubation period, molecular diagnostic methods have become preferable during recent years (Dorman et al., 1983; Sachse et al., 2010; Gioia et al., 2016). Detection of *M. bovis* in bulk tank milk samples (**BTMS**) or cow composite milk samples (**CMS**) by using quantitative PCR (**qPCR**) allows an identification of udder pathogens, including *M. bovis*, rapidly, with high sensitivity, and without previous isolation of bacteria

Received November 7, 2016.
Accepted April 14, 2017.
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(Ghadersohi et al., 1997; Ghadersohi et al., 1999; Fox et al., 2005). Identification of infected herds is best made by analyzing BTMS. *Mycoplasma bovis* positive result indicates that the pathogen is introduced to the herd. However, the true within-herd prevalence cannot be estimated with only a positive BTMS result. Further identification of infected cows should be made by analyzing CMS (Fox et al., 2005).

Although mycoplasmas were identified in North America and Europe decades ago, the prevalence of *M. bovis* mastitis is not widely studied (Fox et al., 2005; Fox, 2012). The herd prevalence of *M. bovis* udder infection ranges between 0.9% in Australia and 1.5% in Belgium (Passchyn et al., 2012; Morton et al., 2014). According to a longitudinal study made in Israel, the number of *M. bovis*-positive dairy herds has increased annually from 2008 to 2014 (Lysnyansky et al., 2016). However, the within-herd prevalence of *M. bovis* mastitis is still not widely studied to this day. By knowing the within-herd prevalence and course of the disease, it could be possible to develop functional control programs and predict new outbreaks. In a study by Murai et al. (2014), a within-herd prevalence of *M. bovis* mastitis was 2.8% (n = 1,210). According to the Estonian Animal Recording Centre database, 19.6% of BTMS (n = 112), analyzed with the PCR method, were positive to *M. bovis* in 2013 (Estonian Animal Recording Centre, 2014).

To our knowledge, no studies are available about the associations between *M. bovis* mastitis and cow milk yield or milk composition. Research about this, however, would clarify the importance of *M. bovis* as an udder pathogen and as a cause of production losses.

The first objective of this study was to identify the within-herd prevalence of subclinical IMI caused by *M. bovis* by qPCR method. The second aim was to find associations between subclinical *M. bovis* infection, cow daily milk yield, SCC, and milk composition.

MATERIALS AND METHODS

Characteristics of the Study Herd

The milk samples were collected from one large, loose-housing dairy herd in Estonia in November 2014. The study herd included 611 dairy cows, of which 89% were Estonian Holstein, and 11% were Estonian Red breed cows. Cows were milked twice a day in the 2 × 12 parallel milking parlors. The average 305-d milk yield was 9,916 kg and bulk milk SCC ranged between 259,000 and 358,000 cells/mL in 2014. *Mycoplasma bovis* was previously detected in BTMS and cow CMS of single cows with clinical mastitis by PCR in 2011.

Collection and Analysis of Composite Milk Samples

The CMS of all 525 lactating dairy cows were collected once during the routine milk recording in November 2014. The daily milk yield of each cow was measured by using a calibrated milk meter (Tru-Test Limited, Auckland, New Zealand). Parity and DIM of all lactating dairy cows were recorded.

All milk samples were preserved with bronopol and transported to the milk laboratory of the Estonian Animal Recording Centre in Tartu. In the laboratory, milk fat (%), protein (%), urea (mg/L), and SCC (× 1,000/mL) were analyzed with accredited methods using the automatic analyzer Combifoss 6000 FC (Hilleroed, Denmark).

After analysis, 1.5 mL of each milk sample was collected and transported to the Estonian University of Life Sciences. Cows with visible signs of clinical mastitis (n = 3) were excluded from the study, and 2 cows without production data were excluded only from the regression analysis. All milk samples (n = 522) were stored at –18°C for further analysis.

qPCR Analysis of Milk Samples

A commercial qPCR test kit Mastit4B (DNA Diagnostic A/S, Risskov, Denmark) was used for qPCR analysis to detect bacterial DNA directly from the milk samples.

The oligos of the Mastitis 4B are designed to detect DNA of *Staph. aureus*, *Strep. agalactiae*, *Strep. uberis*, and *M. bovis*. After thawing, the milk samples were vortexed and from each sample, 500 μL of milk was used for DNA extraction before PCR analysis according to the instructions (http://dna-diagnostic.com/files/Downloads/Mastit4/Instruction_protocol_M4B_2017.04.26.pdf) from the manufacturer (DNA Diagnostic, Risskov, Denmark). The PCR mixture consisted of 15 μL of the qPCR Master Mix and 5 μL of purified DNA. The real-time PCR instrument thermal cycler Stratagene Mx3005P (Agilent Technologies Inc., Santa Clara, CA) was used for amplification. The amplification conditions were as follows: 95°C for 1 min, 1 cycle; 95°C for 5 s and 60°C for 25 s, 40 cycles. Cycle threshold (**Ct**) values were reported for all samples. For all bacteria identified in the analysis, a Ct value of ≤37.0 was considered a positive result. The assay included controls for the validation of each run including negative DNA extraction controls, internal amplification standard (positive PCR controls), and nontemplate control. The assay was validated on both bacterial strains and milk samples by DNA Diagnostic. According to the internal validation protocol of the laboratory, the sensitivity