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Elimination of selected mastitis pathogens during the dry period

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ABSTRACT

We aimed to evaluate the elimination of 4 different mastitis pathogens, *Streptococcus agalactiae*, *Mycoplasma bovis*, *Staphylococcus aureus*, and *Streptococcus uberis*, from infected udder quarters during the dry period using quantitative PCR. The second purpose of this study was to evaluate the association between milk haptoglobin (Hp) concentration and the presence of udder pathogens (*Strep. agalactiae*, *Staph. aureus*, *M. bovis*, and *Strep. uberis*) in udder quarter milk samples before and after dry period. Aseptic udder quarter milk samples (n = 1,001) were collected from 133 dairy cows at dry off and at the first milking after calving from 1 large dairy herd. Bacterial DNA of *Strep. agalactiae*, *Staph. aureus*, *Strep. uberis*, and *M. bovis* in the udder quarter milk samples was identified with commercial quantitative PCR analysis Mastitis 4B (DNA Diagnostic A/S, Risskov, Denmark). Milk Hp concentration (mg/L) was measured from udder quarter milk samples. The elimination rates during the dry period for *M. bovis*, *Staph. aureus*, *Strep. agalactiae*, and *Strep. uberis* were 86.7, 93.6, 96.2, and 100.0%, respectively. The new IMI rate was 3.0% for *M. bovis*, 2.9% for *Staph. aureus*, 2.4% for *Strep. agalactiae*, and 3.1% for *Strep. uberis*. The milk Hp concentration was significantly higher in udder quarter milk samples with blood and in samples positive for *Strep. agalactiae* at dry off and for *Staph. aureus* postcalving. Elevated milk Hp concentration was not associated with the presence of *M. bovis* in the udder quarter milk samples. In conclusion, elimination of *Staph. aureus*, *Strep. agalactiae*, and *Strep. uberis* during the dry period was high; the elimination of *M. bovis* from infected udder quarters was lower, but probably spontaneous. Additionally, milk Hp concentration may be used as a marker for udder inflammation when combined with the bacteriological results at dry off and postpartum.

Key words: udder pathogens, dry period, elimination, haptoglobin

INTRODUCTION

Mastitis is one of the major concerns in dairy herds because it causes economic losses to the dairy industry due to lower milk yield and reduced milk quality (Hertl et al., 2014). Cow mammary glands are more susceptible to the invasion of udder pathogens at dry off and around calving. Natural defense mechanisms, such as lactoferrin, leukocytes, and keratin plug at the teat end, are present in the mammary gland, inhibiting the invasion and growth of udder pathogens during the dry period. To enhance the elimination of udder pathogens from infected udder quarters, dry cow antibiotic therapy alone or together with internal teat-sealants is used at dry off (Bradley and Green, 2004). Usually, treatment against *Streptococcus agalactiae* is effective during the dry period, but recovery from *Staphylococcus aureus* is more difficult; however, some strain differences affect the elimination of *Staph. aureus* in dry cows (Dingwell et al., 2006). Dry cow therapy is considered ineffective in the elimination of *Mycoplasma bovis* from infected udder quarters (Ruegg and Erskine, 2015). Traditionally, the presence of udder pathogens before and after calving has been identified with culture-based methods, but molecular methods, such as PCR, have become more common in detecting udder pathogens from milk samples worldwide (Koskinen et al., 2010).

The mammary environment during the dry period is not advantageous for udder pathogens, and acute clinical mastitis rarely occurs in dry cows (Bradley and Green, 2004). However, subclinical IMI during the dry period serves as a risk factor for clinical mastitis after calving (Bradley and Green, 2004). Mastitis-causing bacteria entering the mammary gland via the teat canal trigger a local inflammatory response and increase the level of acute phase proteins in milk (Pyörälä, 2003). Haptoglobin (**Hp**), one of the acute phase proteins, is mainly produced in the liver, but local production of Hp in mammary gland occurs as Hp is released from

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the damaged epithelial cells and neutrophils of udder tissues (Hiss et al., 2004). Milk Hp concentration can be used as a marker for the detection of udder inflammation (Pyörälä, 2003), as both clinical and subclinical IMI induce elevated milk Hp concentrations (Nielsen et al., 2004; Kalmus et al., 2013).

The elimination rates of *Staph. aureus*, *Strep. agalactiae*, and *Strep. uberis* during the dry period (Bradley et al., 2015) and the associations between these udder pathogens and milk Hp concentration (Eckersall et al., 2006; Pyörälä et al., 2011) have been previously described. No published studies have examined the elimination of *M. bovis* during the dry period. Additionally, associations between *M. bovis* and local inflammatory response in the mammary glands measured through milk Hp concentration have not been studied to our knowledge. The aim of our study was to evaluate the elimination of *Strep. agalactiae*, *Staph. aureus*, *M. bovis*, and *Strep. uberis* from infected udder quarters during the dry period using quantitative PCR (**qPCR**) method. The second aim of our study was to evaluate the association between milk Hp concentration and the presence of udder pathogens (*Strep. agalactiae*, *Staph. aureus*, *M. bovis*, and *Strep. uberis*) in udder quarter milk samples at dry off and postcalving.

MATERIALS AND METHODS

Study Design

Cow aseptic udder quarter milk samples were collected once at dry off and from the first milking after calving between November 2014 and May 2015 from 1 Estonian dairy herd. All the cows that were dried off and calved during that period were sampled.

All the collected udder quarter milk samples (n = 1,001) were analyzed with qPCR for the detection of bacterial DNA of *Staph. aureus*, *Strep. agalactiae*, *M. bovis*, and *Strep. uberis*. Based on the results of the qPCR analysis, the elimination rates and new infection rates were calculated. After qPCR analysis, milk Hp concentration (mg/L) was measured from all collected udder quarter milk samples to evaluate the associations between the presence of udder pathogens and milk Hp concentration.

Characteristics of the Study Herd

The milk samples were collected from 1 Estonian large loose-housed dairy herd from northeastern Estonia. *Mycoplasma bovis* was previously identified in bulk tank milk samples and cow composite milk samples in clinical mastitis cases in 2013. The study herd included 611 dairy cows, of which 89% were Estonian Holstein and 11% Estonian Red. Cows were milked twice per day in a 2 × 12 parallel milking parlor. The average 305-d milk yield was 9,916 kg, and the bulk milk SCC ranged between 259,000 and 358,000 cells/mL in 2014. All cows were treated with cloxacillin-based dry cow antibiotic product (Noroclox DC, 600 mg, Norbrook Laboratories Limited, Newry, Ireland) at dry off. The length of the dry period ranged between 37 and 94 d (median 65 d). Cow parity, DIM, and the length of the dry period were recorded from the database of Estonian Livestock Performance Recording Ltd. (Tartu, Estonia).

Collection of Udder Quarter Milk Samples

Cow udder quarter milk samples were collected at dry off and at the first milking postpartum. Before collection, the teat end was cleaned with 70% ethanol swabs and allowed to dry. After discarding a few streams of milk, samples (2 to 4 mL) were collected into sterile 10-mL plastic tubes. Milk samples were stored at −18°C and transported to the DNA Diagnostic A/S laboratory for further analysis.

qPCR Analysis of Udder Quarter Milk Samples

Bacterial DNA from *M. bovis*, *Staph. aureus*, *Strep. agalactiae*, and *Strep. uberis* was detected by commercial quantitative qPCR test kit Mastitis 4B (DNA Diagnostic A/S). 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 and 500 μL of milk was used for DNA extraction before PCR analysis according to the manufacturer's instructions (DNA Diagnostic A/S, http://dna-diagnostic.com/files/Downloads/Mastit4/Instruction_protocol_M4B_2017.11.01.pdf). 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 95°C for 1 min for 1 cycle, and 95°C for 5 s and 60°C for 25 s for 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 the DNA Diagnostic.