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Changes in Pathogen Number during Preservation of Milk Derived from Mastitic Dairy Cows

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ABSTRACT: Pathogens are sometimes undetected in mastitic milk after cultivation. It was found that several innate immune components such as antimicrobial peptides exist in milk and that their concentration increases in mastitic milk. Therefore, we hypothesized that pathogen in milk is killed by innate immune components during preservation of milk. The present experiment was undertaken to confirm this hypothesis. Milk was collected from mastitic udders (somatic cell count > 300,000 cells/ml) of dairy cows in the southern region of Japan. After preservation of milk at room temperature for 0, 0.5, 1, 2, 3, 4 and 5 h, milk was put on media and cultured for 18 to 48 h to count formed colony. *Streptococci* was detected in 40% of milk and 10% of them contained *Streptococcus uberis*. Coliform and *Staphylococcus aureus* were observed in less than 20% and 10% of milk, respectively. Coagulase negative Staphylococci, Yeast-like fungus and *Corynebacterium bovis* were also detected in less than 10% of milk. The number of *Staphylococcus aureus* in milk has not changed significantly during 5-h cultivation. The number of *Streptococcus uberis* in milk decreased slightly compared with that at 0 h, but there was no significant difference. In the milk with Coliform, number ratio was significantly decreased to under 50% at 4 h compared with that at 0 h. Number ratio was significantly decreased at only 0.5 h of culture in milk with Coagulase negative Staphylococci, Yeast-like fungus, *Corynebacterium bovis* and their ratio further declined at 5 h of culture to under 20% in Coagulase negative Staphylococci or 10% in Yeast-like fungus and *Corynebacterium bovis*. These results suggest that pathogenic microbes in high-somatic cell count milk decreased during preservation at room temperature. Therefore, reduction of microbes from the time of collection to examination should be taken into consideration to evaluate milk contamination.

Keywords: Dairy cow, Somatic cell count, Milk, Preservation, Pathogen

INTRODUCTION

Mastitis is an inflammatory condition of the udder in bovine and other species caused by bacterial infection. It reduces milk production, with consequent economic losses for the dairy industry. However, approximately 10–40% of clinical mastitis cases yield "no significant growth" in routine clinical culture assays, although the reason for this is currently unknown (Östensson *et al.*, 2013; OldeRiekerink *et al.*, 2008). This may be due to infection that has been bacteria present in low numbers, even though the SCC has not yet decreased. Other considerations include sampling procedure, treatment of milk samples, methods, and media used in the bacteriological examination, the presence of pathogens below current detection thresholds, the absence of the bacteria at the time culture is initiated, or that the mastitis may be caused by non-bacterial microorganisms (Hogan *et al.* 1999; Kuehn *et al.*, 2013). Since selection of antibiotics largely depends on the infecting bacterial species, it is important to clarify the reason why the milk was diagnosed as negative for pathogens.

Many kinds of antimicrobial components (one of the innate immune factors) such as lingual antimicrobial peptide (LAP), cathelicidins, lactoferrin (LF), lactoperoxidase (LPO) and S100 protein are produced in mammary epithelial cells and leukocytes and secreted into milk (Swanson *et al.* 2004; Isobe *et al.*, 2009, 2011; Regenhard *et al.* 2010; Tetens *et al.* 2010). Their concentration in milk is increased in mastitic milk compared with that in healthy milk (Isobe *et al.,* 2009; Edwin *et al.,* 1977; Kawai *et al.*, 2013; Morimoto *et al.*, 2012; Zhang *et al.*, 2014). Therefore, the milk from mastitic cows contains a high amount of antimicrobial components.

It takes at least several hours from milking to culturing in medical centers. During this intervening period, it may be possible that pathogens in milk are killed by the antimicrobial components. However, this possibility remains to be elucidated. Therefore, the objective of the present study was to investigate the change of the number of living pathogens during the preservation of milk collected from mastitic udder.

MATERIALS AND METHODS

Sixty-two Holstein Friesian cows were used. California mastitis test (CMT) in the quarter milk was performed before collection and only CMT-positive milk was collected. SCC of milk was measured by fluorescence optics type somatic cell measuring equipment (Somscope series, Milestone-General, KAWASAKI). Milk with SCC of >300,000 cells/ml was considered as subclinical mastitis. Other parts of the mastitic milk were kept at room temperature for 0, 0.5, 1, 2, 3, 4 and 5 h. Then, 50 µl of the milk was plated onto 5% sheep blood agar, and cultured at 37°C for 18 to 48 h to count colony forming unit (CFU). The identification of the pathogen was conducted using the usual method.

RESULTS AND DISCUSSION

Streptococci was detected in 40% of milk and 10 % of them contained *Streptococcus uberis*. *Coliform* and *Staphylococcus aureus* were observed in less than 20% and 10% of milk, respectively. Coagulase negative Staphylococci, Yeast-like fungus and *Corynebacterium bovis* were detected in less than 10% of milk. The number of Staphylococcus aureus in milk has not changed significantly during 5-h cultivation. The number of Streptococcus uberis in milk decreased slightly at 0.5 and 1 h compared with that at 0 h, but there was no significant difference. In the milk with Coliform, number ratio was significantly decreased to under 50% at 4 h compared with that at 0 h. Number ratio was significantly decreased at only 0.5 h of culture in milk with Coagulase negative *Staphylococci*, Yeast-like fungus and *Corynebacterium bovis* and their ratio further declined at 5 h of culture to under 20% in Coagulase negative Staphylococci or under 10% in Yeast-like fungus and *Corynebacterium bovis.*

These results suggest that some pathogens in high-somatic cell count milk decreased during preservation at room temperature. Therefore, reduction of microbes from the time of collection to examination should be taken into consideration to evaluate milk contamination.

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