



## Longitudinal study of the effects of teat condition on the risk of new intramammary infections in dairy cows

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### ABSTRACT

Machine milking-induced alterations of teat tissue may impair local defense mechanisms and increase the risk of new intramammary infections. The objective of the current study was to assess the influence of short-term and long-term alterations of teat tissue and infectious status of the udder quarter on the risk of naturally occurring new intramammary infections, inflammatory responses, and mastitis. Short-term and long-term changes in teat condition of right udder quarters of 135 cows of a commercial dairy farm in Saxony-Anhalt, Germany, were recorded monthly for 10 mo using simple classification schemes. Quarter milk samples were collected from all examined quarters at each farm visit. Bacteriological culture results and somatic cell counts of quarter milk samples were used to determine new inflammatory responses (increase from  $\leq 100,000$  cells/mL to  $> 100,000$  cells/mL between 2 samples), new infections (detection of a pathogen from a quarter that was free of the same pathogen at the preceding sampling), and new mastitis (combination of new inflammatory response and new infection). Separate Poisson mixed models for new inflammatory responses, new infections, and new mastitis caused by specific pathogens or groups of pathogens (contagious, environmental, major, minor, or any) were used to estimate risk ratios and 95% confidence intervals. Data preparation and parameter estimation were performed using the open source statistical analysis software R. We observed no effect of any variable describing teat condition on the risk of new intramammary infections, inflammatory responses, or mastitis. Intramammary infections of the same udder quarter in the preceding month did not affect risk either.

**Key words:** mastitis, udder health, hyperkeratosis, teat edema, risk factor

### INTRODUCTION

Mastitis is one of the most common health disorders in dairy cows (Egger-Danner et al., 2012). Infections with pathogenic bacteria entering the mammary gland via the teat canal are the most frequent cause of mastitis (O'Shea, 1987; GVA, 2012). Therefore, the condition of the teat as a barrier against infection is considered a major factor influencing the frequency of inflamed udder quarters (Mein, 2012). Machine milking-induced tissue alterations at the teat may impair local defense mechanisms (O'Shea, 1987; Mein, 2012). Mein et al. (2001) distinguished between short-term and long-term changes in teat condition. Short-term changes in teat condition are determined by tissue responses to a single milking such as congestion and edema. After cluster detachment, the teats should be dry, smooth, and pink (Krömker, 2007). Long-term changes in teat condition are the adaptation of the teat tissue to machine milking over weeks and are mainly defined by the degree of teat-end hyperkeratosis. Mein (2012) suggested that short-term changes inhibit timely closure of the teat canal orifice after milking and increase the risk of new intramammary infections (NIMI) by contagious as well as environmental mastitis pathogens. Due to the rough surface of a hyperkeratotic teat canal, more mastitis-causing bacteria can be found on the teat ends of teats with long-term changes. Such pathogens can more easily enter a teat canal with hyperkeratosis than a teat canal without hyperkeratosis (Neijenhuis et al., 2001b). A simple classification scheme to evaluate changes in teat condition for on-farm use has been developed (Mein et al., 2001). The evaluation includes color changes of the teat, swelling at the teat base and teat end, and callosity of the teat end. Only a few studies have investigated relationships between short-term changes in teat condition and mastitis (Binde and Bakke, 1984; Zecconi et al., 1992; Zwertvaegher et al., 2013). Of these, only one studied naturally occurring NIMI as the dependent variable: Zecconi et al. (1992) could not show an association between changes in teat

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diameter as a measure of edema and infections with environmental pathogens. The results of observational studies evaluating associations between long-term changes in teat condition and mastitis are ambiguous (Haverkamp and Krömker, 2010).

Studies looking for associations between teat condition and NIMI are rare (Zadoks et al., 2001). However, reducing NIMI is crucial for improving udder health (Neave et al., 1969; Reyher et al., 2013). Therefore, evidence-based information about risk factors for NIMI is essential. Causal inference is only possible if the time series of occurrences can be clearly defined (Reyher et al., 2013). Longitudinal studies with repeated determinations of infectious status are needed to identify probable causes for NIMI but they are time consuming (Zadoks et al., 2001). The objective of the present study was to investigate possible associations between short-term and long-term changes in teat condition, infectious status, and naturally occurring NIMI with different pathogens and new inflammatory responses under field conditions in a longitudinal design.

## MATERIALS AND METHODS

### *Herd and Animals*

The study was conducted from May 2009 to February 2010 on a commercial dairy farm in Saxony-Anhalt, Germany. The farm kept, on average, 800 German Holstein cows housed in freestall barns. The cows were milked twice a day on a rotary parlor with 40 milking units and automated cluster removal (GEA Farm Technologies GmbH, Bönen, Germany). The milking machine was routinely checked according to ISO 5707:2007 (ISO, 2007) once a year. The farm was selected because of a high monthly incidence of udder inflammations based on DHI data in the preceding year (i.e., every month above the regional median of 21%). Mean milk yield in 305 lactation days was 10,410 kg of ECM. Mean bulk milk SCC was 215,000 cells/mL at the beginning of the study.

All cows between 15 and 90 DIM at the first visit were included in the study unless they met one or more of the following exclusion criteria: cows that had already been treated because of mastitis in their current lactation, cows that had a SCC of more than 250,000 cells/mL in the last DHIA test before initiation of the study, or cows that had fewer than 4 functional udder quarters. Cows with black teats on the right udder half were excluded to ensure accurate determination of teat color changes (Mein et al., 2001). Only the 2 right teats were examined because a careful examination of all 4 teats was not possible without disrupting the milking

routine. All cows included in the study were examined monthly for 10 mo or until they were dried-off, sold, or culled.

### *Data Collection*

During the 10 monthly visits, short-term and long-term changes in teat condition were determined, and quarter milk samples were taken by 2 trained investigators at the usual milkings of the herd. Illustrated scorecards were used, and teat condition of the first 5 cows was evaluated by the investigators together at first visit to obtain concordant results. Last calving date and current lactation number of the selected cows were taken from the herd management software.

**Teat Condition.** Short-term and long-term changes in teat condition were determined within 1 min of removal of the milking clusters according to Mein et al. (2001). Teats were examined visually and by manual palpation. Color changes of the teat skin were categorized as “pink,” “reddened,” or “blue.” Swelling at or near the teat base was recorded as “normal” (mild swelling or slight lip mark of the liner mouthpiece) or as “ring” (palpable ring where the teat tissue had swollen into the liner mouthpiece chamber). The teat end was classified as “normal” (soft tissue without palpable swelling) or “swollen.” Teat-end hyperkeratosis was described as no ring, a smooth ring, a rough ring, or a very rough ring around the teat orifice. The condition of every teat was documented, together with cow identification number, teat position (front right or hind right), and date of the farm visit.

**Milk Samples.** Quarter foremilk samples of the 2 right udder quarters of all cows included in the study were taken at each farm visit aseptically according to the guidelines of the German Veterinary Association (GVA, 2009) after routine udder preparation and disinfection of the teat ends with 70% alcohol. Udders were routinely prepared by farm personnel by milking of 2 or 3 streams per teat and cleaning the teats with spin-dried cotton cloths. Separate cotton cloths were used for each cow. Approximately 10 mL of milk was sampled into sterile plastic tubes with preservative Ly20 containing boric acid (Heeschen et al., 1969; GVA, 2009) and analyzed within 24 h in the microbiological laboratory of the University of Applied Science and Arts (Hannover, Germany).

Somatic cell counts of all milk samples were analyzed using flow cytometry (SomaScope Smart, Delta Instruments, Drachten, the Netherlands). Culturing of the milk samples and identification of the isolates were carried out according to National Mastitis Council and German Veterinary Association recommendations

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