Reduction of udder microbial contamination during milking routines*

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ABSTRACT

Milking hygiene includes pre- and post-milking routines as well as the cleanliness of milking equipment. Standard udder hygiene procedures consist of pre-dipping, dry wiping, fore-stripping, cleaning or drying of teats, and post-dipping of teats. The use of effective preparations and procedures for the sanitary treatment of the cow udder significantly reduces contamination of the teat skin, thereby reducing the overall bacterial contamination of milk. The aim of the study was to evaluate the effectiveness of disinfection of teats and teat cups in reducing the microbial load before milking on two dairy cow farms with different milking hygiene regimes. The regime on the first farm consisted of teat soaking with foam and mechanical cleaning with disinfectant wipes before milking, while udder hygiene on the second farm consisted in the use of disinfectant wipes alone, without foaming. On both farms, the results showed a reduction in the microbial load of the teats and teat cup after disinfection. The reduction in the microbial load was greater on the first farm than on the second, owing to pre-milking dipping in foam with lactic acid as the main active ingredient together with disinfection of teat cups with hydrogen peroxide. This resulted in milk that was less contaminated and thus safer for human consumption.

KEY WORDS: cows, milking, teats, disinfection, contamination

Introduction

Infected cows and a contaminated environment in the milking parlour are potential sources of food-borne pathogens and spoilage bacteria, which affect milk quality and present a public health risk. Environmental hygiene and treatment of dairy cows are two of the most important components of programmes for controlling mastitis. A sanitary environment reduces the occurrence of pathogens, while the main effect of therapy is an increased rate of elimination of infections. Both of these practices significantly reduce the spread and transmission of bacteria and subsequent intramammary infection [2]. Anything that comes into contact with an infected udder is a potential source of pathogens, so it is important to adhere to the established milking programme [12].

Pre-milking procedures may consist of soaking, foaming, cleaning, or drying teats. [11]. The use of effective preparations and facilities for sanitary treatment of the udder of dairy cows significantly reduces contamination of the teat skin and reduces the overall bacterial contamination of milk [8]. Many types of disinfectants are used for teat soaking, such as iodine chlorhexidine, acidified sodium chlorite, peroxide, and organic acids (lactic acid, salicylic acid, capric acid, or glycolic acid). Inadequate hygiene procedures, improper mechanical cleaning, the use of inappropriate disinfectants, insufficiently clean equipment, the microbial load in the air, a defective water supply, and poor housing conditions can all significantly affect the health of the mammary gland and contribute to contamination of raw milk [13].

The most common contaminants of milking equipment include bacteria such as *Escherichia coli*, *S. aureus*, *Listeria monocytogenes*, *Salmonella* spp., *Micrococcus* spp., *Campylobacter jejuni*, *Enterococcus faecalis*, coagulase-negative staphylococci, *Citrobacter freundii*, and others [3].

The microbial load of raw milk is influenced by microorganisms present in the teat canals and on the skin surface of the mammary gland. The teat surface has been identified as the largest contributor to microbial contamination of raw milk, followed by faeces [13, 15]. This is consistent with studies by Vargová et al. [12] and Reugg [9], who reported that teat skin was the primary source of microbial contamination of raw milk and that the rate of intramammary infections increases with the number of bacteria on the teat skin and on the

milking equipment. The aim of this study was to compare two different procedures for cleaning and disinfecting teats and teat cups to reduce the microbial load before and after milking on dairy cow farms.

Material and methods

Dairy herds and milking methods

The practical part of the study was carried out on two dairy farms located in eastern Slovakia, with 250–350 cows of the Slovak Spotted Cattle breed, between their 1st and 4th lactation, with an average daily milk yield of 21.6 ±2.4 L and 23.2 ±3.1 L. The cows were fed a TMR diet based on silage, hay and concentrate in accordance with nutritional requirements for milk yield of 20–30 L per day [7]. On both farms, cows were milked in the morning and in the evening, following different milking hygiene programmes.

Milking hygiene programme on the first farm

The preparation Prefoam + (Hypred S.A., Dinard, France), intended for teat soaking, was used at the start of the milking procedure. Prefoam+, with the active biocidal component 5% L-(+)-lactic acid, was applied as a foam for udder hygiene before milking. After foaming, the first squirts of milk were collected into a container with a double bottom, and the teats were mechanically cleaned with UdderClean wet wipes (Agromont, Nitra), intended for cleaning the entire udder. UdderClean wipes have several advantages. They are used to clean the udder, stimulate milk production, shorten milking time, and clean the milker's hands. The milking and pulsation vacuum was set to 42 kPa. The pulsation ratio was 60:40 at a speed of 52 c/min, and the end of milking was signalled automatically when the milk flow dropped to 0.2 L/min. After milking, the ends of the teats were disinfected with a solution containing lactic acid as the active ingredient. As an important intermediate step, the teats were washed and disinfected with 2% hydrogen peroxide after each change of cow group in the milking parlour. The milk was stored in cooling tanks at +4°C until it was collected the next day.

Milking hygiene programme on the second farm

On the second farm, preparation for milking consisted in cleaning the udder with wet wipes soaked in DERMISAN+ disinfectant solution (Agromont Nitra, SR, active substance: 15,000 mg/kg N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine) to remove dirt from the udder and teats, without additional foaming and wiping. Subsequently, the first squirts of milk were drawn with the milking unit. The technical parameters of milking and pulsation were set as on the first farm, with automatic termination of milking when the milk flow dropped below 0.2 L/min. After milking, teats were disinfected by soaking in Ioderm 5000 disinfectant (Agromont Nitra, SR, active substance: 5000 mg/kg iodine). Teat cups were washed only with water during milking, without disinfectants. The milk was stored in cooling tanks at +4°C until it was collected the next day.

Collection and examination of samples

The surfaces of 15 dairy cows and milking equipment from each farm were sampled. Two teat skin samples were taken from each dairy cow. For the teat samples, swabs were taken before milking and after foaming (the second farm did not perform pre-milking teat foaming) or after mechanical cleaning. Teat cup samples were taken three times: before milking, after milking, and after disinfection with hydrogen peroxide or washing with water. The samples were tested in the laboratory according to Vargova et al. [2].

For total bacterial count (TBC) and coliform bacteria (CBC), swab samples were diluted in sterile saline. Diluted solutions (volume 0.1 ml) were then plated using the casting method on the selective medium Endo agar (EA; HiMedia, India) and Nutrient agar no. 2 (NA; HiMedia, India) according to established procedures [6]. TBC was determined according to ISO 18593:2004, and CBC according to ISO 4832:2006 [4, 5]. Endo agar and Nutrient agar results were obtained after 24 h of incubation at 37°C.

Data analysis

The TBC and CBC obtained from the teat and teat cup swabs were converted to decimal logarithms ($\log_{10}$ CFU/cm^2), and analysis of variance (ANOVA) was performed. Data were analysed using Graph Pad Prism software, and $p < 0.05$ was assumed to indicate a significant difference.

Results and discussion

The teat skin of cows is the primary source of bacterial populations found in raw milk, with the rate of mastitis and intramammary infections having previously been shown to increase with bacterial numbers on the teat skin [9]. Many bacterial *strains have been associated with mastitis, mai*nly *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Escherichia coli* [3, 14].

Parameters such as somatic cell count and the total number of microorganisms are used to assess the health safety of raw milk. To monitor microbial contamination and environmental hygiene, swab sampling methods are used for cultures to determine the total number of bacteria and coliform bacteria [13].

Evaluation of the microbial load after foaming of the teats and cleaning with disinfectant wipes on the first farm showed a significant decrease ($p < 0.05$) in TBC and CBC compared to their state before cleaning. On

Table 1

Influence of the sanitation regime on the microbial load of the teat skin (log_{10} CFU/cm²)

	Before cleaning		After cleaning[1]		After milking[2]	
	TBC	CBC	TBC	CBC	TBC	CBC
Farm 1	4.36	2.33	0.98	0.92	0.16	0.1
Farm 2	4.81	2.54	2.05	1.45	0.1	0.1
P value	NS	NS	p<0.05	p<0.05	NS	NS

After cleaning[1] – on the first farm, the teats were cleaned with a foamer and wet wipes; on the second farm, the teats were cleaned only with wet wipes. After milking[2] – on the first farm, the teat ends were disinfected after milking with a solution containing lactic acid as the active ingredient; on the second farm, the teat ends were disinfected with an iodine solution. TBC – total bacterial count, CBC – coliform bacteria, CFU – colony forming units

the second farm, where the teats were not soaked and only wet wipes were used, the reduction in the microbial load (TBC and CBC) on the teat skin was smaller ($p < 0.05$). Comparison of the effectiveness of the two hygiene regimes showed that soaking teats plays a significant role in their disinfection and in reduction of the microbial load. A combination of foaming and mechanical cleaning of the teats with wet wipes eliminated microbial contamination on the farm more efficiently (Table 1).

According to Suriyasathaporn and Chupia [10], disinfection of the teats and teat cups reduces the bacterial load on the teat skin and also reduces the risk of bacterial contamination of milk. In some studies, the concentration of microorganisms such as *Staphylococcus aureus* or coagulase-negative staphylococci obtained by swabbing the teat skin was lower after dipping of teats in a disinfectant solution after milking, compared to untreated teats. The reduction in the bacterial load on teat skin can have a positive impact by minimizing contamination of milk [1, 12]. The results of our study indicate an important intermediate step, i.e. disinfection of teats during milking, which significantly reduces the transmission of contagious pathogens to the milk as well as from cow to cow. Using hydrogen peroxide to disinfect teats during the milking process on the first farm significantly reduced TBC and CBC compared to the second farm, where the teats were only rinsed with hot water (Table 2). These results indicate that on the second farm higher microbial contamination remained on both the teats and teat cups following the udder hygiene procedures.

Vargova et al. [12] detected 44 samples positive for *S. aureus* (42% of the total number of samples), which was the most common pathogen from the evaluated surfaces. *S. aureus* was identified from teats, teat cups, and wipes used for mechanical cleaning of the udder. Therefore this study indicates that teat dipping and teat cup disinfection plays a significant role in reducing the microbial load.

Table 2

Influence of the sanitation regime on the microbial load of the teat cups (log_{10} CFU/cm²)

	Before milking		After milking		After cleaning[1]	
	TBC	CBC	TBC	CBC	TBC	CBC
Farm 1	1.55	0.9	2.09	1.22	0.7	0.55
Farm 2	1.5	0.85	2.25	1.35	1.0	0.85
P value	NS	NS	NS	NS	p<0.05	p<0.05

After cleaning[1] – on the first farm teat cups were disinfected with 2% hydrogen peroxide after each change of cow group in the milking parlour; on the second farm they were rinsed only with water. TBC – total bacterial count, CB – coliform bacteria, CFU – colony forming units

Conclusion

This study emphasizes the importance of strict hygiene programmes in order to reduce microbial contamination of the skin of the udder and milk equipment. Our results indicate that treating the udder with pre-milking foam with lactic acid as the main active ingredient in addition to disinfectant wipes is more effective for reducing bacteria than treating the teat with disinfectant wipes alone. Therefore it can be concluded that teat dipping plays a significant role in reducing the microbial load and should be performed on every teat before milking. In addition to cleaning the udder, it is recommended to disinfect the milking unit and teats with hydrogen peroxide followed by rinsing with water to reduce the microbial load and thus obtain high-quality milk that will be safer for consumption.

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Zmniejszenie skażenia mikrobiologicznego wymienia podczas doju

Streszczenie

Higiena doju obejmuje zarówno procedury przed, jak i po doju, a także sprzętu używanego podczas doju krów. Rutynowe czynności obejmują mycie, dezynfekcję strzyków, osuszenie i wykonanie post-dippingu. Stosowanie skutecznych preparatów i środków do higieny wymion krów znacznie zmniejsza poziom patogenów na skórze strzyków, ograniczając tym samym ogólne zanieczyszczenie bakteryjne mleka. Celem pracy była ocena skuteczności dezynfekcji strzyków i kubków udojowych przed dojem w ograniczaniu obciążenia mikrobiologicznego. Badania zostały przeprowadzone na dwóch fermach krów mlecznych o zróżnicowanych standardach higienicznych doju. W pierwszym stadzie wykonywano przed dojem kąpiel strzyków preparatem w formie piany z zawartością 5% L-(+) - kwasu mlekowego, następnie wymię wycierano chusteczkami dezynfekującymi. W drugim gospodarstwie wykonywano dezynfekcję wymion jedynie przy użyciu chusteczek dezynfekcyjnych, bez piany. Uzyskane wyniki wskazują, że w obu gospodarstwach po dezynfekcji nastąpiła redukcja poziomu patogenów na strzykach i kubkach udojowych. W pierwszym stadzie uzyskano wyższy poziom redukcji drobnoustrojów dzięki zastosowaniu przed udojem pianki z 5% kwasem mlekowym jako głównym składnikiem aktywnym, przy jednoczesnym wykorzystaniu wody utlenionej do dezynfekcji kubków udojowych, co skutkowało możliwością pozyskania surowca o niższej zawartości drobnoustrojów, bezpiecznego dla konsumenta.

SŁOWA KLUCZOWE: krowy, dój, strzyki, dezynfekcja, skażenie