The efficacy of a foaming iodine-based pre-milking teat disinfectant

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Abstract

Pre-milking teat disinfection is practised in several countries to prevent environment-related mastitis. This study was designed to prove the antimicrobial efficacy of a foaming, iodine-based teat disinfectant with five different concentrations (250; 500; 1,000; 2,000 and 3,000 ppm) against a negative control. For this purpose the split-udder design was used and within an udder two teats were dipped with the test product before milking, while the other two teats were left untreated. After the customary udder preparation (pre-milking, cleaning of the teats with dry paper towel) the teat skin's microbial load was investigated using the wet and dry swab technique. The total bacterial count, the counts of streptococci and streptococci like organisms (SSLO) and coliform bacteria were analysed. The associations between the treatment of the teats and the microbial load were analysed with a linear mixed regression model for repeated measurements. Microbial load with considered microorganisms was significantly lower on the skin of teats disinfected before milking compared to teats that were only cleaned. There are no differences in efficacy between the tested concentrations, e.g. the 250 ppm teat disinfectant was as effective as the 3000 ppm teat disinfectant.

Keywords: udder hygiene, teat skin, microbial load reduction, iodine

Introduction

The microbial load of the teat skin consists of a physiological flora and facultative pathogenic microorganisms. The physiological flora is represented by species/genera like *Corynebacterium*, *Bacillus*, *Aerococcus*, *Acinetobacter*, *Psychrobacter*, *Staphylococcus* (*Staph.*), *Enterococcus*, *Pediococcus*, *Enterobacter* and *Pantoea* [1, 2]. These physiological microorganisms influence each other and the growth of potentially pathogenic microorganisms [1, 3, 4]. The colonisation of the teat skin with cow-associated and environment-related pathogenic microorganisms is described as a potential starting point for the invasion into the bovine mammary gland [5, 6]. Thereby, the largest populations are built by *Streptococcus* (*Strep.*) *uberis* and coliforms as environmental pathogens [6]. The bacterial counts of *Strep. uberis*, *Escherichia (E.) coli* and other coliform bacteria on the teat skin are associated with the treatment of the bedding material: The counts are significantly lower when the bedding material is treated with an alkaline conditioner in comparison to no treatment of the bedding material [28]. This association could not be shown for cow-associated pathogens like *Staph. aureus* [28]. Furthermore, Paduch et al. [6] demonstrated a positive correlation between the counts of pathogens on the teat skin and in the teat canal. Concerning teat end hyperkeratosis and the colonisation with pathogens, there is also a positive correlation between the hyperkeratosis score and the findings for *E. coli* as well as for the microbial load of *E. coli* and *Strep. uberis* in the teat canal [27].

Pre-milking teat disinfection is practised in several countries to reduce the microbial load of the teats prior to milking and to prevent mastitis caused by environmental pathogens. In Germany, pre-milking teat disinfection is not a common used practice, because of several reservations regarding residues of the disinfectant in the milk. Nonetheless, the teat disinfection with a product licensed to use prior to milking is in Germany allowed by statutory provisions.

Several disinfectants like hypochlorite, chlorine dioxide, chlorhexidine, alcohol, dodecyl-benzol-sulfonic acid and iodine reduce the microbial load of the teats significantly [7, 8, 9, 10]. The rate of new intramammary infections and the incidence of clinical mastitis caused by environmental pathogens like *E. coli* and *Strep. uberis* are significantly lower when pre-milking teat disinfection is practised [11, 12, 13, 14, 15]. The rate of new intramammary infections caused by cow-associated pathogens like *Staph. aureus* and potentially pathogenic microorganisms like coagulase negative Staphylococci (CNS) and *Corynebacterium* (*C.*) bovis is not affected by pre-milking teat disinfection [11, 12, 15, 16].

This study was conducted to prove the efficacy of a pre-milking, foaming, iodine-based teat disinfectant in reducing the microbial load of the teats in comparison to a negative control. Furthermore, the efficacy differences between several iodine concentrations in the teat disinfectant should be evaluated to discover the lowest effective iodine concentration. The lowest effective iodine concentration in the pre-milking teat disinfection should be evaluated to reduce the risk of residues in the raw milk.

Material and Methods

Herd and animals:

The study was conducted on a commercial dairy farm with 100 cows in the German federal state North Rhine-Westphalia. The cows were housed in a free-stall barn equipped with raised bedded cubicles with sawdust as well as with deep bedded cubicles with ground straw. The

cows were milked twice a day in a double six herringbone milking parlour. Usually no pre-milking teat disinfection was practised. The milkers were always wearing gloves. After milking, all teats were dipped with a non-film-building product containing lactic acid and chlorine dioxide of which no residues were visible on the teats to the next milking time. 25 lactating cows without clinical mastitis and four functioning quarters (no swelling of the udder, no fever, no flakes in the milk) were used for sample collection. Furthermore, the cows had no teat skin lesions. All samples were taken at the same milking place so that the cows were sampled as they came into the parlour, i.e. they were not preselected.

Pre-milking teat disinfectant:

For this study, a foaming teat disinfectant based on a fatty acid ethoxylate iodine-complex at concentrations of 250, 500, 1,000, 2,000 and 3,000 ppm iodine was tested. These disinfectants were produced as prototypes by the Ferdinand Eimermacher GmbH & Co KG in Nordwalde, Germany. The disinfection concentrations were ready-to-use solutions.

Udder preparation:

To evaluate the efficacy of the five different concentrations of the pre-milking teat disinfectant the split-udder design was used. Two of the teats of an animal (either front left and hind right or front right and hind left, alternating) were dipped once with the product before milking using a conventional foam dip cup. With this foam dip cup, 0.4 mL of the disinfectant was applied to each treated teat. The other two teats were used as a negative control. For each concentration group the same disinfectant solution was used. For every concentration of the teat disinfection product five cows were used for the sample collection. After an exposure time of 30 seconds, the first streams of milk were manually milked and rejected, beginning with the untreated teats to minimise the transfer of the disinfectant. This resulted in a total exposure time of 35 seconds. After that all teats were cleaned with one dry paper towel per cow.

Sample collection:

After cleaning the teats, the samples were taken using the wet and dry swab technique in accordance with DIN 10113-1: 1997-07. For this, a cotton wool swab (ultrafine, dry swab, 30MW113, Check Diagnostics, Germany) moistened with sterile 0.25 % Ringer's solution (Merck, Germany) was moved around the teat at a distance of 1 cm from the teat canal orifice. After that the same procedure was performed with a dry cotton wool swab (ultrafine, dry swab, 30MW113, Check Diagnostics, Germany). Both swabs were shortened and inserted into one test tube containing 2 mL of the sterile 0.25 % Ringer's solution [6, 27].

Microbiology:

After collection, the samples were stored at 5°celsius and transported within 2 hours to the microbiological laboratory of the University of Applied Sciences and Arts Hannover, Germany. The tips of the cotton wool swabs were vortexed with a mixer for 20 seconds in the Ringer's solution and removed with aseptic forceps afterwards. With this swab solution a serial dilution in accordance with § 64 LFGB, method: L 00.00 54 was generated. The agar plates were inoculated in duplicate with 0.1 mL of the swab solution and the serial dilutions (10^{-1}, 10^{-2}).

For evaluating the total bacterial count, plate count agar plates (Merck, Germany) were used, *E. coli* and other coliform bacteria were detected using ChromoCult coliform agar plates (Merck, Germany). The counts of streptococci and streptococci like organisms (SSLO) including *Strep. uberis* were determined using a modified Edwards medium containing colistin sulfate (5 mg/L) and oxolinic acid (2.5 mg/L) [17].

The modified Edwards agar plates and the ChromoCult coliform agar plates were incubated aerobically at 37°C for 24 h. The esculin-positive colonies on the modified Edwards agar were counted as esculin-positive SSLO. Colonies on the ChromoCult agar plates were counted as coliforms including *E. coli*.

Plate count agar plates were incubated aerobically at 30°C for 72 h.

The results from plates with 1 - 300 colonies were used to calculate bacterial counts in the teat skin swab solution. The weighted arithmetic means were calculated for each investigated pathogen group (total bacterial count, SSLO and coliforms). Results were reported as colony-forming units per mL of swab solution (cfu/mL).

Statistics

To achieve statistical normal distribution the bacterial counts were logarithmised to base 10 after adding 1 ($\log_{10}$ cfu/mL) and SPSS 23.0 software (IBM, USA) was applied for data analysis. Descriptive statistics (mean, standard error of the mean, minimum, maximum) were calculated. For each pathogen group, subdivided by the concentration of the disinfectant, the associations between the treatment of the teats and the microbial load were analysed with a linear mixed regression model for repeated measurements. The subject was the teat pair. Statistical significance was defined as $p < 0.05$. Furthermore the additional reduction rates based on the total counts of pathogens caused by the predipping compared to the normal udder preparation were calculated.

Results

For this study, 100 teat skin swab samples were taken from 100 quarters of 25 lactating dairy cattle. Thereby 50 teat pairs were used for the analysis. All cows were free of clinical mastitis and had no teat skin lesions.

Total bacterial count:

The mean total bacterial counts after cleaning of the teats with a dry paper towel, calculated for all five concentrations, amounted to 2.92 ± 0.559 $\log_{10}$ cfu/mL (Table 1). After disinfection and cleaning of the teats the mean of total bacterial counts amounted to 2.12 ± 0.593 $\log_{10}$ cfu/mL (Table 1). Therefore, the additional reduction rate was 84.15 % and the effect of the disinfection was significant with $p < 0.0001$. There were no significant differences between the five tested iodine concentrations in reducing the total microbial load of the teat skin ($p = 0.673$).

Streptococci and Streptococci like organisms (SSLO):

The mean SSLO count for all untreated teats included in this study was 2.38 ± 0.361 $\log_{10}$ cfu/mL; for the teats, which were treated with a disinfectant, the mean of SSLO counts amounted to 1.39 ± 0.945 $\log_{10}$ cfu/mL (Table 1). The additional teat disinfection resulted in a significant reduction in the teat skin colonisation with SSLO ($p < 0.0001$). The additional reduction rate reached 89.77 %. Thereby, no significant differences between the five concentrations were detected ($p = 0.155$).

***E. coli* and other coliforms (CC):**

The mean count of *E. coli* and other coliforms on the teats after standard cleaning amounted to 0.25 ± 0.587 log10 cfu/mL (Table 1). After disinfection the mean count of *E. coli* and other coliforms on the teats was 0.02 ± 0.146 $\log_{10}$ cfu/mL (Table 1). Henceforth, the disinfection resulted in an additional reduction rate of 41.12 % and thereby was significant with $p = 0.009$. There were no significant differences between the five tested iodine concentrations ($p = 0.132$).

Discussion

The present study was conducted to evaluate the efficacy of pre-milking teat disinfection in reducing the microbial load on the teat skin compared to normal udder preparation. Furthermore, the

Table 1: Mean bacterial counts on the teats in cfu/ml ± standard error of the mean

	log_{10} total bacterial counts		log_{10} *SSLO*		log_{10} coliforms	
	untreated	treated	untreated	treated	untreated	treated
250 ppm	2.82 ± 0.846	1.81 ± 0,370	2.21 ± 0.995	1.29 ± 0.501	0.34 ± 0.525	0.00
500 ppm	2.96 ± 0.321	2.13 ± 0.554	2.94 ± 0.652	1.71 ± 0.991	0.58 ± 0.959	0.00
1,000 ppm	3.00 ± 0.407	2.30 ± 0.717	2.54 ± 0.824	1.40 ± 1.188	0.34 ± 0.525	0.10 ± 0.312
2,000 ppm	2.94 ± 0.183	2.16 ± 0.486	2.08 ± 0.361	0.98 ± 0.809	0.00	0.00
3,000 ppm	2.89 ± 0.725	2.19 ± 0.657	2.13 ± 0.757	1.56 ± 0.922	0.00	0.00
Sum of all 50 teat pairs	2.92 ±0.559	2.12 ± 0.593	2.38 ± 0.361	1.39 ± 0.945	0.25 ± 0.587	0.02 ± 0.146

influence of the iodine concentration in the disinfectant on the antimicrobial effectiveness was evaluated. Therefore, five teat disinfectants containing identical ingredients except for the iodine concentration were tested under the same conditions, e.g. on the same farm, in the same milking parlour and under identical climate conditions. This was necessary to get comparable results about the efficacy of the different iodine concentrations. Otherwise there might be biasing influences by different ingredients in the disinfectants, different housing conditions or different climate conditions.

The disinfectant used was a foaming dip which was applied to the teats with a conventional foaming dip cup and removed after a total exposure time of 35 seconds with a dry paper towel.

In general, the exposure time depends on the active ingredient and the formulation of the product. However, in order to fit into the milking routine, the pre-milking udder preparation has to be done quickly and therefore the exposure time has to be as short as possible. Several former studies showed that an exposure time of 30 seconds results in good antimicrobial effects [8, 18, 19, 20, 10]. Enger et al. [10] showed for a 0.25 % and 1.0 % iodine dip that an exposure time of 30 seconds was as effective as an exposure time of 45 seconds. Reducing the exposure time to 15 seconds resulted in lower reduction rates.

The disinfectant was applied prior to fore-milking in order to expand the contact time. Another possible procedure might have been to start with fore-milking, then applying the disinfectant and cleaning the teats afterwards, but this might possibly result in too short contact times.

When applying the disinfectant, independent from the concentration, the reduction in the tested microorganisms was significantly higher compared to standard cleaning of the teats. Former studies led to comparable results [8, 9, 21]. Galton et al. [21] tested three teat dips, containing iodine, hypochlorite and dodecyl-benzol-sulfonic acid, which were used prior to milking. All dips reduced the total microbial counts on the teats significantly, there also being no differences between the active ingredients. Ingawa et al. [8] evaluated that a cleaning gel and a 0.5 % iodine dip significantly reduced the microbial load of teat ends. In contrast to this study, Gleeson et al. [9] found no significant differences between the use of iodine, chlorine, chlorhexidine or alcohol for pre-milking teat disinfection and 'no treatment' or 'washing and drying of the teats' in reducing the load of the teat skin with coliform bacteria. Nonetheless, they found a significant difference for the reduction in staphylococci and streptococci.

Comparing the five different iodine concentrations of the used teat disinfectant (250; 500; 1,000; 2,000 and 3,000 ppm), no significant differences in reducing the microbial load of the teat skin were found. This can be explained by the balance of the available iodine and the free iodine in a solution. As iodine is insoluble in water it always has to be bound in a complex [22]. In a solution, there is always a part of free iodine which has been released from the complex. This free iodine is responsible for the antimicrobial efficacy. The content of free iodine in a solution depends on the total available iodine content [23, 24]. The content of free iodine in the tested solutions is nearly the same between 250 and 3,000 ppm available iodine. Thereby, the same efficacy of the five tested concentrations can be explained. Both, a lower and a higher iodine concentration would not be of benefit as the concentration of free iodine would decrease [23, 24].

The counts of coliform bacteria are lower than expected. Especially in case of the deep bedded cubicles without alkaline conditioner the coliform counts would be expected to reach higher levels. Paduch et al. [28] examined that the mean coliform counts on the teat skin is 1.4 ± 0,2 log_{10} cfu/mL when using untreated sawdust for bedding material. In this study it was 0.25 ± 0.587 log_{10} cfu/mL. Nonetheless, as iodine causes the denaturation of proteins and is well known for its efficacy against all kinds of microorganisms it can be expected that iodine kills coliforms, if available, as effectively as other microorganisms [22].

The additive reduction rate caused by pre-milking teat disinfection deviates between 41 % and 89 %. This appears to be quite a wide range, but as the coliform counts were generally low in this study, the 41 % reduction rate of coliforms may not be representative. The reduction rates of SSLO and the total bacterial counts (89.77 % and 84.15 %, respectively) indicate that pre-milking teat disinfection may reduce the risk of new intramammary infections caused by environment-related pathogens. To show this effect, further studies with the tested disinfectant would be needed. These studies should include larger numbers of cows and possibly evaluate the disinfection effect directly at the teat duct orifice concerning the effects of hyperkeratosis and the effectiveness of pre-milking teat disinfection in the field. Former studies already showed for other disinfectants that pre-milking teat disinfection is an effective tool to reduce the new intramammary infection rate with environmental pathogens like *Strep. uberis*, *Strep. dysgalactiae* and coliforms [14, 15, 25, 26].

Conclusion

The present study shows that all five concentrations reduce the microbial load of the teat skin significantly. As there are no significant differences between the iodine concentrations, it can be recommended to use low dosages of iodine in pre-milking teat disinfection to minimise the risk of iodine residues in raw milk.

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