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Effect of a pre-milking teat foam and a liner disinfectant on the presence of mesophilic and (proteolytic) psychrotrophic bacteria prior to milking

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Research Article

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Abstract

Contamination of raw milk by psychrotrophs can lead to the production of heat-resistant proteases and subsequent spoilage of UHT milk. Therefore, this research communication evaluated the effect of a pre-milking teat disinfectant (active components: L-(+)-lactic acid and salicylic acid) and a liner disinfectant (active components: peracetic acid and hydrogen peroxide) on the number of mesophilic and (proteolytic) psychrotrophic bacteria prior to milking. The teat orifices of 10 cows were sampled using a swabbing procedure before and after treatment with a pre-milking teat disinfectant on six subsequent days. On the teat orifices, there was a small but statistically significant decrease in the psychrotrophic bacterial counts between pre and post dipping. No differences were observed for the mesophilic bacterial counts and proteolytic active counts. Liners were also sampled using swabs pre and post disinfection. No statistically significant decrease in the bacterial counts was observed post liner disinfection, although there was a numerical decrease. Sixty-two percent of the proteolytic psychrotrophs were pseudomonads: 16.5% of which were *P. fragi*, 14.3% *P. lundensis*, 10.0% *P. fluorescens* and 2.9% *P. putida*. Trinitrobenzenesulfonic acid (TNBS) analysis revealed a wide variety in proteolytic activity (from 0 to 55 µmol glycine/ml milk) and the presence of high producers. It can be concluded that there was only a minor effect of teat and liner disinfection on the psychrotrophic bacterial counts indicating that the measures presented did not result in a reduction of the targeted bacteria on teat orifices and liners.

Psychrotrophic bacteria and pseudomonads in particular can produce heat-resistant proteases during refrigerated storage of raw milk. These proteases are able to endure the high temperatures used for UHT treatment of milk, which may result in enzymatic milk degradation possibly reducing product shelf life. This phenomenon has important economic consequences for dairy producers (De Jonghe *et al.*, 2011).

To reduce the growth of psychrotrophic bacteria, the most common measure is to store the raw milk at a temperature below 4 °C. This measure is not always easy to perform in the dairy production chain (De Jonghe *et al.*, 2011). Therefore, preventing or reducing the number of psychrotrophs and pseudomonads entering the raw milk at the farm could be a useful control measure. Pseudomonads are widespread throughout the farm environment and are present on udders and the liners of the milking machine (Nucera *et al.*, 2016). Disinfectants used for teat dipping or liner disinfection are available and are frequently used for maintaining udder health. To our knowledge, the effect of such products on (proteolytic) psychrotrophic bacteria and on pseudomonads in particular has not yet been determined. Our objective was to evaluate the effect of two disinfectants, one a 'foam' type pre-milking teat disinfectant applied as a dip (pre-dip) and the other a liner disinfectant, on the mesophilic and (proteolytic) psychrotrophic bacterial counts. Additionally, we identified the proteolytic active pseudomonads and determined their proteolytic activity.

Materials and methods

Ten cows from the experimental dairy herd of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Melle, BE) were chosen based on their parity and the absence of mastitis: three from first parity, three from second parity, four from third parity or higher. The experimental setup for the pre-dip teat disinfectant and liner disinfectant is described in the Supplementary File. Briefly, swabs were taken from the cow's teat orifice (±0.5 cm^2) pre and post disinfecting the teats with a teat pre-dip foam (Prefoam +, Hypred SA,

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Table 1. Overview of the bacterial count averages before (pre) and after (post) disinfection of the teats or liners

	Bacterial counts averages log cfu/ml teat or liner suspension ± std dev				
	TEATS (Day 1 to 9)			LINERS (Cup 1 to 14)	
Sample (pre or post disinfection)	Mesophilic	Psychrotrophic	Proteolytic Psychrotrophic	Mesophilic	Psychrotrophic
1 – pre	3.65 ± 0.43	3.54 ± 0.70	2.00 ± 0.00	3.18 ± 0.41	1.64 ± 0.82
1 – post	NA	NA	NA	3.45 ± 0.84	2.09 ± 0.96
2 – pre	4.31 ± 0.96	2.94 ± 1.17	1.94 ± 0.82	2.58 ± 0.93	2.15 ± 0.21
2 – post	NA	NA	NA	3.25 ± 0.27	1.72 ± 0.39
3 – pre	4.30 ± 0.69	3.38 ± 0.36	2.00 ± 0.00	3.69 ± 0.97	2.36 ± 0.54
3 – post	NA	NA	NA	2.16 ± 1.13	1.41 ± 0.35
4 – pre	3.60 ± 0.42	3.19 ± 0.42	1.80 ± 0.28	2.64 ± 1.18	1.99 ± 0.41
4 – post	4.32 ± 0.97	3.30 ± 0.78	1.91 ± 0.58	3.01 ± 0.89	1.58 ± 0.51
5 – pre	4.44 ± 1.12	4.35 ± 0.99	1.00 ± 0.00	2.26 ± 1.16	1.92 ± 0.11
5 – post	4.22 ± 0.90	3.65 ± 0.95	1.70 ± 0.00	2.68 ± 1.27	1.94 ± 0.14
6 – pre	4.61 ± 0.69	3.32 ± 0.46	1.15 ± 0.21	3.87 ± 1.69	2.49 ± 1.03
6 – post	4.26 ± 0.85	2.83 ± 0.40	1.42 ± 0.46	2.97 ± 1.14	1.87 ± 0.59
7 – pre	3.55 ± 0.44	3.40 ± 0.38	1.00 ± 0.00	2.29 ± 1.68	2.84 ± 0.90
7 – post	4.14 ± 0.60	3.33 ± 0.33	1.44 ± 0.97	2.94 ± 0.58	1.95 ± 0.14
8 – pre	3.82 ± 1.30	3.43 ± 0.43	1.48 ± 0.43	3.95 ± 0.78	1.97 ± 0.69
8 – post	3.72 ± 0.54	3.26 ± 0.52	0.00 ± 0.00	3.22 ± 0.20	1.30 ± 0.00
9 – pre	4.25 ± 0.87	3.22 ± 0.34	1.60 ± 0.43	3.42 ± 0.68	2.03 ± 0.84
9 – post	4.34 ± 0.74	3.23 ± 0.44	1.30 ± 0.00	3.89 ± 1.82	1.93 ± 0.66
10 – pre				3.76 ± 1.00	1.48 ± 0.00
10 – post				2.95 ± 0.69	1.93 ± 0.73
11 – pre				2.79 ± 1.03	1.60 ± 0.00
11 – post				2.60 ± 0.78	1.90 ± 0.00
12 – pre				2.48 ± 1.48	1.48 ± 0.00
12 – post				2.72 ± 0.59	1.85 ± 0.00
13 – pre				3.38 ± 0.56	1.84 ± 0.28
13 – post				3.81 ± 0.88	2.23 ± 0.04
14 – pre				3.12 ± 2.12	3.16 ± 0.00
14 – post				2.39 ± 1.02	2.29 ± 0.48
Average pre	4.06 ± 0.40	3.42 ± 0.39	1.55 ± 0.42	3.15 ± 0.55	2.07 ± 0.50
Average post	4.17 ± 0.22	3.27 ± 0.26	1.29 ± 0.67	3.00 ± 0.50	1.86 ± 0.28

NA, not available.
The teats were sampled for nine consecutive days of which three days served as a control period. There were 14 liners that were sampled before and after disinfection.

Dinard, France; active components: L-(+)-lactic acid and salicylic acid) and from the liners (±5 cm^2) pre and post treating the liners with a disinfectant spray (Perfo Grif, Hypred SA, Dinard, France; active components: peracetic acid and hydrogen peroxide).

The mesophilic and (proteolytic) psychrotrophic counts in log cfu/ml teat or liner suspension were determined on all samples ($n = 206$). The pseudomonads were identified to species level using PCR methodology and the proteolytic activity was determined using the trinitrobenzenesulfonic acid (TNBS) method (Marchand, 2009). Statistical analyses were performed in SAS (Supplementary File).

Results and discussion

In the control period, mesophilic and psychrotrophic bacteria were found in all teat orifice samples, but proteolytic active psychrotrophs in only 30% of the samples. The daily average mesophilic count was 4.06 log cfu/ml teat suspension with a SD of ±0.40 cfu/

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ml. Other authors found mesophilic counts of 5–6 log cfu/ml teat suspension, which is remarkably higher (Verdier-Metz *et al.*, 2012; Guarin *et al.*, 2017). However, although this was not mentioned actually in the reports, the swabbed teat surfaces seemed more than 10 times larger than in our study, which may explain the observed differences. The daily average psychrotrophic count was 3.42 ± 0.39 log cfu/ml. The daily average proteolytic count was 1.55 ± 0.42 log cfu/ml throughout the experiment (Table 1).

The milking team noticed that cows' udders change over time: younger cows have a smoother teat skin and the teat canal closes more tightly in comparison to older cows. A rougher teat skin will be more difficult to clean during pre-milking teat preparation which could result in a site more prone to bacterial colonization (Guarin *et al.*, 2017). To counter these differences, cows from three parities were selected, but no statistically significant differences between the parities' bacterial counts were observed.

Following the control period (days 1–3), teats were then prepared using the foam pre-dip from days 4–9. The daily average bacterial counts (log cfu/ml teat suspension) pre and post using the foam pre-dip are shown in Table 1. There was no statistical difference pre and post the treatment for the mesophilic counts ($P = 0.63$), but there was a statistical difference for the psychrotrophic counts ($P < 0.01$) (Table 1). However, this difference should be put in perspective: a difference in bacterial count that is less than one log is generally not considered to be biologically relevant. There were no significant differences between the parities after disinfection. In some cases, an increase in bacterial counts was observed after disinfection. This could be due to applying different pressures to the swab when sampling (Fitzpatrick *et al.*, 2018).

It has been suggested that an effective teat disinfectant should minimally yield a 3 log reduction in bacterial numbers, with a 4–5 log reduction being preferable (Fitzpatrick *et al.*, 2018). We did not observe more than a 3 log reduction in bacterial numbers in our study for either mesophilic or psychotrophic counts. This implies that the foam pre-dip did not affect the number of pseudomonads and subsequently is most unlikely to reduce pseudomonad contamination of the raw milk.

According to the manufacturer, the main targets of the foam pre-dip are *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus uberis*, bacteria which are of importance for udder health. The foam pre-dip solution did not result in any biologically relevant decreases in the mesophilic and (proteolytic) psychrotrophic counts. However, there was removal of the visual dirt after use, resulting in visually cleaner teats which could result in a lower risk of infection and thus a better udder health. In this study we only sampled cows from one herd and used only one pre-dip disinfectant, so confirmation of these results and evaluation of other pre-dip formulations is required.

Milking machine liners were also swabbed after rinsing (before disinfection) and after disinfection. The averages of the mesophilic and psychrotrophic counts pre and post disinfection of each milking cluster (4 liners per device) are shown in Table 1. The average mesophilic bacterial counts were 3.15 ± 0.55 and 3.00 ± 0.50 log cfu/ml liner suspension for pre and post liner disinfection, respectively. Thirty-nine of the 56 (22 before and 17 after disinfection) samples tested below the detection limit for the psychrotrophic counts (<1 log cfu/ml). The average psychrotrophic counts of the remaining samples was 2.07 ± 0.50 and 1.86 ± 0.28 log cfu/ml liner suspension pre and post disinfection, respectively. No proteolytic active psychrotrophs were found. Again, there was no significant effect of the disinfection on the mesophilic ($P = 0.46$) and psychrotrophic ($P = 0.18$) bacterial counts. According to the manufacturer's manual this liner disinfectant is bactericidal for udder pathogens, such as *S. aureus*, *E. coli* and *S. uberis*, as the foam pre-dip is. Our study only tested the disinfection once prior to milking; more investigation with more disinfection rounds is required to confirm these results. In addition, it would be interesting to investigate combinations of measures (disinfecting both teats and liners).

Throughout the study, 227 proteolytic psychrotrophic isolates were obtained. Of these, 140 (61.7%) were identified as pseudomonads, the predominant species found in raw milk after cold storage (De Jonghe *et al.*, 2011). These comprised 16.5% *P. fragi*, 14.3% *P. lundensis,* 10.0% *P. fluorescens* and 2.9% *P. putida*. The remaining isolates were other *Pseudomonas* species. This is in contrast to the study of Nucera *et al.* (2016), where 45% of the proteolytic active pseudomonads in dairy environment were identified as *P. fluorescens*. However, these authors used another and probably less discriminatory method to identify the pseudomonads than the one that we used, which could explain the observed difference.

The proteolytic activity of these pseudomonads ranged from 0 to 55.42 µmol glycine equivalents/ml milk. The majority of the *P. fragi* and *P. lundensis* isolates showed proteolytic activity within a range of 0.05 to 55.42 µmol glycine/ml. Approximately half of the *P. fluorescens* isolates had proteolytic activities ranging from 0.04 to 3.95 µmol glycine/ml. Three *P. putida* isolates had a very low proteolytic activity (0.09–0.14 µmol glycine/ml). In general, there is a huge variation in proteolytic activity within one species. Marchand (2009) found proteolytic ranges of 0 to 10.98 µmol glycine/ml within one species. Notably, 'high' protease producers (16 out of 140) were observed with a proteolytic activity up to 55 µmol glycine/ml; to our knowledge this is the first report of this phenomenon. Should these high protease producers express such a high proteolytic activity during refrigerated storage of raw milk, we estimate that this would result in degradation of the UHT-treated milk within one month. Normally, the proteolytic activity in bulk tank milk is lower than 1 µmol glycine/ml (internal communication ILVO).

In conclusion, we tested a foam pre-dip and liner disinfectant and did not find the expected reductions in bacterial counts. There was a statistically significant effect of the pre-dip on the psychrotrophic bacterial counts although the biological relevance of this reduction in bacterial numbers is questionable. The measures presented in this study to reduce pseudomonads did not result in a reduction of the targeted bacteria on teat apices and liners. Of the proteolytic isolates obtained showing a broad range of proteolytic activity, 61.7% were identified as pseudomonads. We also identified high protease producers which have not been previously described.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919000700

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