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The reduction in bacterial counts on liners by flushing with the Airwash system

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Contents

1. Introduction

Pathogens may be transferred from cow to cow by the liners during milking and thereby compromise the udder health. Backflushing of liners is a method to reduce or eliminate transfer of bacteria. Disinfectants in the flush water ensure low bacterial counts but the risk of residues in the milk is not negligible. Consequently, there is a need for efficient flushing systems without the risk of contamination of the milk. The Airwash system flushes liners in between milking of each cow by the use of compressed air and water. Earlier results with flushing by the Airwash system were disappointing (Eriksen & Rasmussen, 1994); flushing reduced total bacterial counts with 50%, coliformes by 68%, and *Staphylococcus aureus* by 61% without having any effect on udder health and cell count. The Airwash system had been improved technically since then and better flushing effects are expected.

The objective of the present experiment was to prove that flushing in between milking of each cow with the Airwash system keeps bacterial counts on liners low compared to no flushing.

2. Material and methods

2.1 EXPERIMENTAL HERD

The commercial herd at Resengård, Trige, with 88 milking Danish Jersey cows was used. Cows were housed in an insulated barn with slatted floor and straw bedded stalls. Cows were on pasture during daytime. The milking parlour was 2 x 7 herring bone with Alfa Laval Harmony clusters (liner no. 99900782). Milking vacuum was 38 kPa and lifting height was 0 cm. Automatic cluster removers were used. In January 1995, about 10% of the cows were infected with *Staphylococcus aureus*.

2.2 THE AIRWASH SYSTEM

The short milk tube was cut 5 cm from the bottom and the Airwash nozzle inserted in the liner through a tube with an inner diameter of 12 mm. Flushing after milking was activated one side at a time, usually as milked cows left the parlour. The flushing sequence consists of 11 periods with water and compressed air. The total amount of flushing water per cluster was adjusted to 750 ml and had a temperature of 28 °C.

2.3 EXPERIMENTAL PROCEDURE

Liner swabs were taken at evening milkings on July 31, August 1 and 3. Side 1 in the parlour was flushed by the Airwash system after milking of a row of cows on the first and last date, and side 2 was flushed at the second with no flushing in between milkings. The liners pointed downwards during flushing.

Liner swabs from left front of the first 5 units (6 units on dates 2 and 3) in each side were taken before milking started and after milking of each cow immediately after flushing (or no flushing). The right front liner was swabbed if left front quarter of the just milked cow was blind.

2.4 SWABBING PROCEDURE

Liners were swabbed inside with sterile cotton swabs in the barrel at either 6.5, 7.0, or 7.5 cm from the mouth piece lip. First swab of a liner on a specific date was done at about 6.5 cm, second swab at 7.0 cm, third swab at 7.5 cm, fourth swab at 6.5 cm and so on. One turn was taken with the cotton swab inside the liner without turning it in the hand. Precaution was taken not to touch any other place of the liner with the cotton swab. The applied pressure to the sides of the liner was about the same for each swab. The cotton swab was moistened in 0.1% proteose peptone before swabbing and broken off in the same tube after swabbing.

2.5 BACTERIOLOGICAL PROCEDURE

Samples were examined for total bacterial counts (SPC) and *Staphylococcus aureus* after standard laboratory methods, table 1.

Table 1. Procedures for bacteriological examination of liner swabs (Culture Media Merck, 1984)

	Standard Plate Count	*Staphylococcus aureus*
Culture medium	Plate Count Agar	Baird-Paker Agar and sulfamethazine
Method	Pour, 1 ml	Surface, 0.1 ml
Dilution	10^{-0}, 10^{-1}, 10^{-2}	10^{-1} and 10^{-2}
Count	All colonies	Black, shiny, convex colonies, 1-5 mm in diameter, white edge surrounded by a clear zone 2-5 mm wide

2.6 QUARTER MILK SAMPLES

Quarter milk samples were taken from all milking cows in the herd one week before swabbing of liners started. Bacteriological status and CMT scores of each quarter were then available. Cell counts of individual cows from the last milk recording were also used in the statistical analyses.

2.7 OTHER RECORDINGS

Individual cow numbers were recorded at each experimental milking. Amount of flush water f0or the swabbed liners was determined at each experimental milking. No symptoms of clinical mastitis during the experiment were detected.

2.8 STATISTICAL PROCEDURES

Bacterial counts (logarithmic values) were tested in the model:

Bact = Airwash + Date + Milking + Infection + Airwash x Date + Airwash x Milking + Airwash x Infection + Airwash x Date x Milking

where

Airwash was the effect of flushing (yes, no)
Date was the effect of date (1, 2, 3)

Milking was the effect of number of milkings (0, 1, ….6) either as regression or as categorial variable.
Infection was the bacteriological status, CMT score or cell count of a specific cow.
Airwash x Date, Airwash x Milking, Airwash x Infection, and Airwash x Date x Milking were the interaction terms.

Total bacterial counts of plates with no growth were set to 0.2 colonies before the logarithmic transformation. Counts of *Staphylococcus aureus* were all added 0.2 before the logarithmic transformation. The procedure General Linear Models (SAS Users Guide, 1989) was used and data are presented as Least Square Means. Independent variables were removed from the model if the level of significance was larger than 10%.

3. Results and discussion

3.1 QUARTER MILK SAMPLES

Samples were taken from 88 cows and 36% of the cows were infected, table 2. Number of *Staphylococcus aureus* infected cows were less than expected and Coagulase Negative Staphylococci were predominant. 20% of the cows had a dry quarter.

3.2 AMOUNT OF FLUSH WATER

Amount of flush water was between 150-190 ml of the swabbed liners except one liner that was flushed with 70-100 ml. The variation in flush water within liner was 5 ml only. The rear liner of the one with the low amount of flush water was flushed with about 250 ml.

Table 2. Bacteriological status of cows and quarters one week before liner swabbing started

	No. of quarters	No. of cows
Staphylococcus aureus	5	5
Coagulase Negative Staphylococci	31	21
Streptococci	8	7
Coliformes and others	5	5
CMT-reaction only	8	6
Dry quarters	18	18
No remarks	277	41

The statistical analysis did not point this front liner out as having higher bacterial counts. Eriksen & Rasmussen (1994) found that an increase in the amount of flush water reduced bacterial counts but amount of flush water per liner was about 40 ml only.

3.3 STATISTICAL MODELS

The best model (R^2 = 0.71, SD = 0.68) describing total bacterial counts was:

Log SPC = Airwash + Date + Milking + Airwash x Date + Airwash x Milking + Airwash x Date x Milking

The independent variables were all significant at the 0.0001 level. The best model (R^2 = 0.12, SD = 0.56) describing numbers of *Staphylococcus aureus* was:

Log *Staphylococcus aureus* = Airwash + Date + Infection + Airwash x Infection

The independent variable 'Airwash' was significant at the 0.05 level and 'Date' at the 0.001 level. Infection and the interaction 'Airwash x Infection' were significant at the 0.01 level. The factor 'infection' was cows with at least one quarter with a CMT reaction or bacteria in a quarter milk sample no matter CMT score. The R^2-value was low for the model with *Staphylococcus aureus* as response and probably because 62% of the samples after no flushing and 66% following flushing had no growth of *Staphylococcus aureus.* Consequently, the statistical procedure GLM may not be valid because of lack of normality. The procedure CATMOD (SAS Users Guide, 1989) was used to test log *Staphylococcus aureus* as a categorial variable in the same model. Independent variables were significant at the same levels as before and predicted means were compatible with those in table 3.

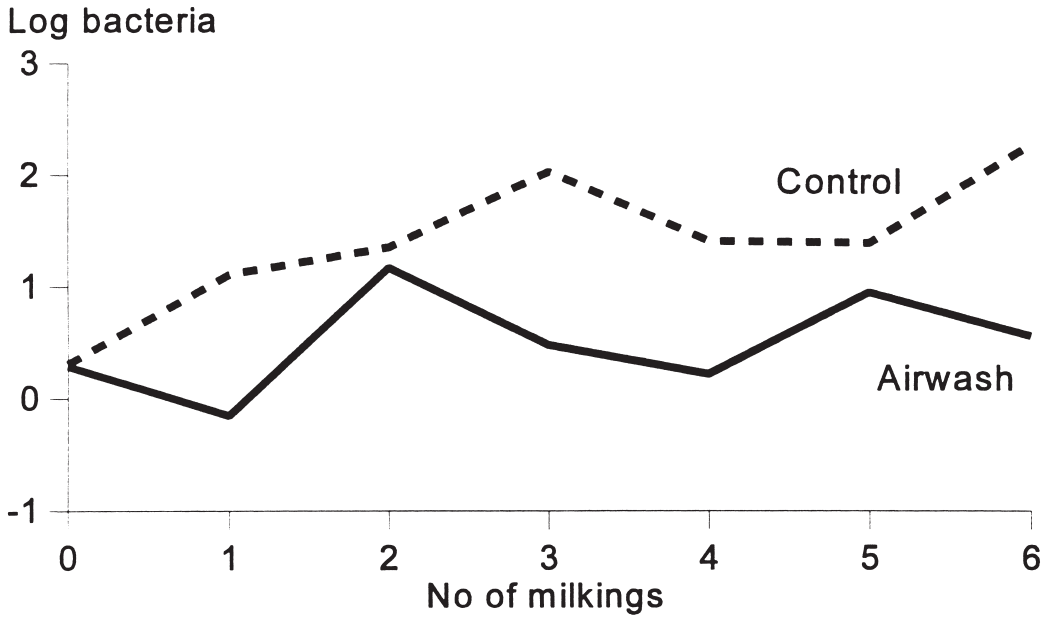

Figure 1. The effect of flushing with the Airwash system between milking of each cow on total bacterial counts on liners.

3.4 THE EFFECT OF FLUSHING

Overall the Airwash system reduced total bacterial counts in liners with 88% ($P<0.001$) and *Staphylococcus aureus* with 48% ($P<0.05$). In general, total bacterial counts increased of unflushed liners during milking ($P<0.001$) whereas bacterial counts remained lower of flushed liners, figure 1.

The total bacterial counts of the control and Airwash groups could be fitted with 2nd degree polynomia ($P=0.08$), but R^2 was lower than by the use of Milking as categorial factor.

Flushing effects differed from day to day ($P<0.0$-01), figures 2-4. Initial bacterial counts were higher and counts fluctuated more on the first day of sampling compared to the last two days. Reductions in total bacterial counts were 38, 93, and 96% of the three days, respectively. The poor result of flushing on day 1 was the main reason for the significant interaction ($P<0.001$) between 'Airwash', 'Date', and 'Milking' (figure 2). Bacterial counts of liner swabs after 2nd and 5th milkings were higher after flushing than without flushing. We have no data that might explain these differences. There was no significant interaction ($P=0.6$) between 'Airwash' and 'Date' of *Staphylococcus aureus* counts i.e. the same effect of flushing by the Airwash system was present every day.

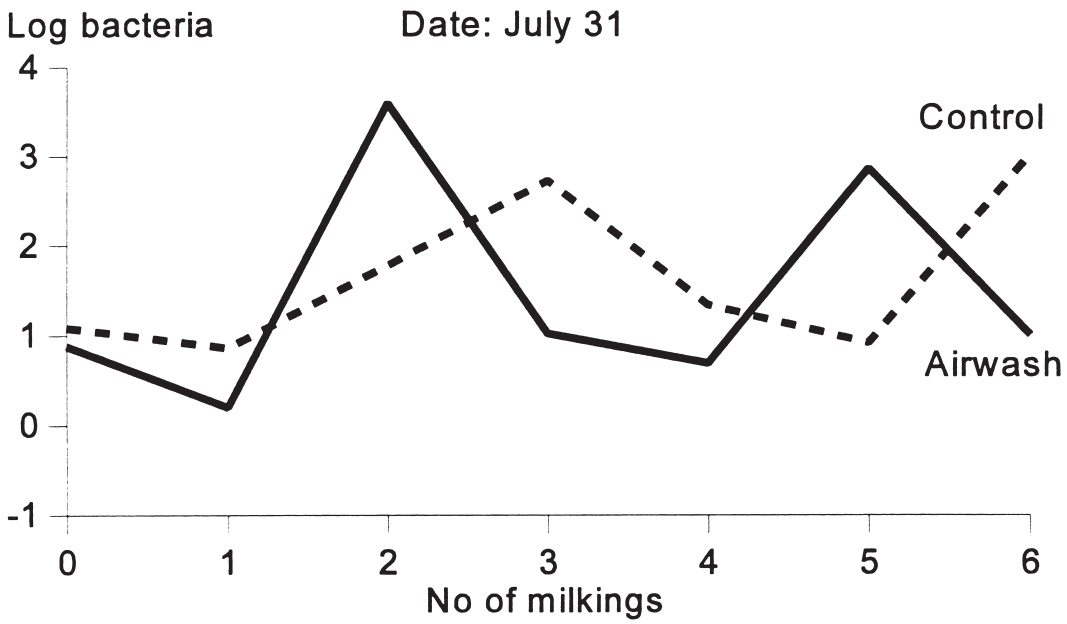

Figure 2. The effect of flushing with the Airwash system between milking of each cow on total bacterial counts on liners on the first day of sampling.

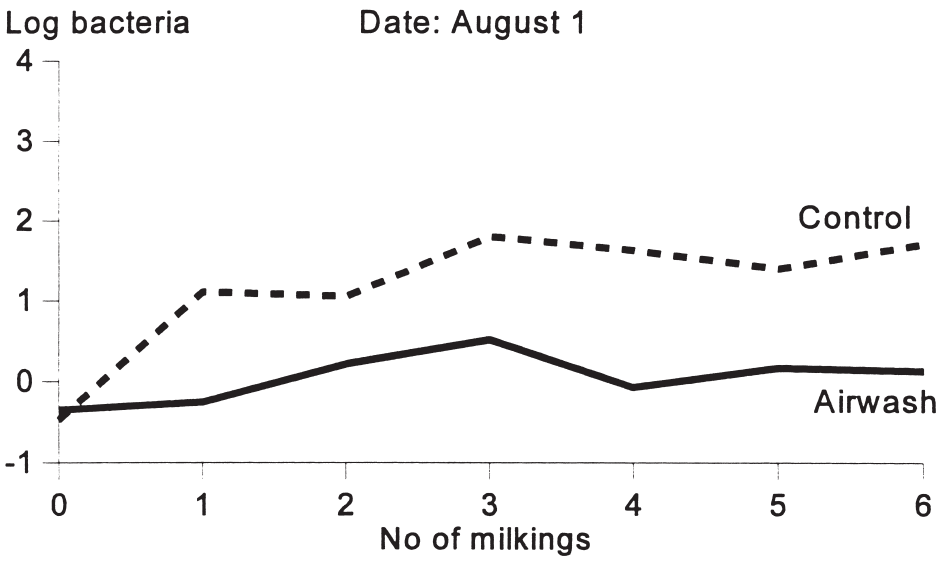

Figure 3. The effect of flushing with the Airwash system between milking of each cow on total bacterial counts on liners on the second day of sampling.

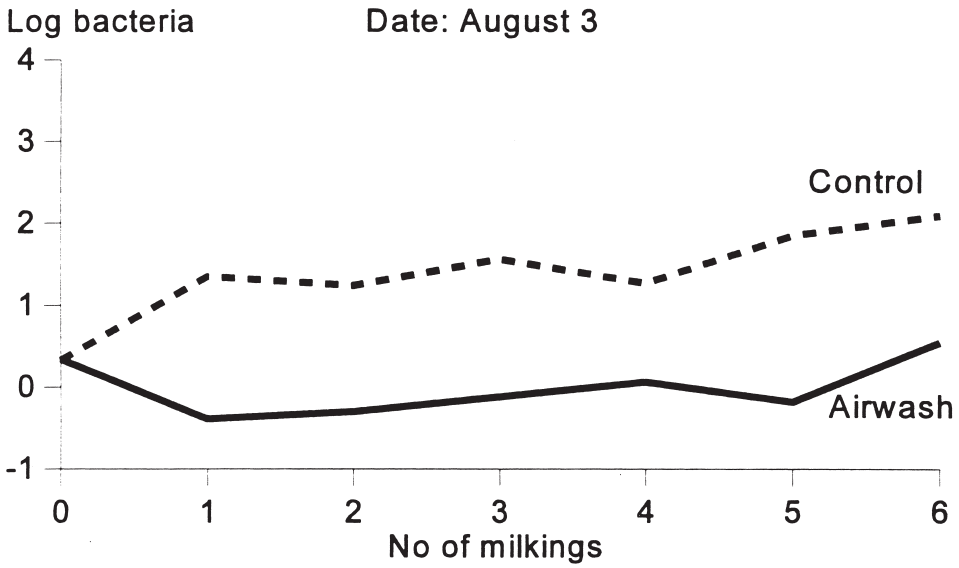

Figure 4. The effect of flushing with the Airwash system between milking of each cow on total bacterial counts on liners on the third day of sampling.

3.5 THE INFLUENCE OF INFECTED QUARTERS ON BACTERIAL COUNTS ON LINERS

The interaction between Airwash and CMT was significant for *Staphylococcus aureus* ($P<0.01$), but not for SPC, table 3. The Airwash system reduced counts of *Staphylococcus aureus* on liners from 9,3 bact/ml tot 2.3 (75%) after milking of infected cows.

Total bacterial counts were higher (not significant) in unflushed liners after milking of infected cows compared to uninfected cows. Bacterial counts were low of flushed liners no matter infectional status of the milked cow. The experiment was not designed to elucidate the effect of reduction in bacterial counts on the udder health.

Table 3. Effect of flushing with the Airwash system on bacterial counts on liners after milking infected (bacteria or CMT reaction in at least one quarter) and uninfected cows

		SPC		*Staphylococcus aureus*	
Airwash	Infected	Log	Bact/ml	Log	Bact/ml
No	No	1.33	21.5	-0.39[a]	2.1
No	Yes	1.60	39.5	0.05[b]	9.3
Yes	No	0.52	3.3	-0.37[a]	2.3
Yes	Yes	0.46	2.9	-0.37[a]	2.3
Significance		0.22		<0.01	

4. Conclusion

Flushing of liners with the Airwash system between milking of each cow reduced total bacterial counts of liners with about 90%.

Flushing reduced *Staphylococcus aureus* counts after milking infected cows with about 75%.

5. Acknowledgements

The technical assistance of E.S. Frimer and M. Linnemann is gratefully acknowledged.

6. Literature

Culture Media Merck. Handbook.

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