

Udder health effects of polyurethane-based external teat sealer

J. Lücking^{1,2}, E.M. Mansion-de Vries^{1,2}, N. Went¹, J.-H. Paduch¹, M. Hoedemaker² and V. Krömker¹

¹University of Applied Sciences and Arts Hannover, Faculty II, Department of Microbiology, Heisterbergallee 12, 30453 Hannover, Germany

²University of Veterinary Medicine Hanover, Clinic for Cattle, Bischofsholer Damm 15, 30173 Hannover, Germany

Date submitted: 02/06/2016

Date accepted: 03/08/2016

Volume/Page(s): 69/38-42

Abstract

Intramammary infections from the prepartum period can affect udder health in the following lactation. The aim of this study was to investigate the effects of a polyurethane-based external teat sealer, which was applied on average 4.4 days before calving to primigravid animals and 2.8 days before calving to multigravid animals, on the development of udder health.

In a split udder study, the teat canal orifices of the front left and the hind right udder quarter of clinical udder-healthy cows were sealed with the external teat sealer when first signs of approaching parturition were observed. Front right and hind left teats were left untreated. Aseptic quarter foremilk samples taken in week 1 and 2 after calving were investigated bacteriologically. Clinical mastitis in the first 100 days of lactation was recorded.

In total, 130 primigravid and 93 multigravid cows were included into the study. In comparison to unsealed quarters (2.9% infected quarters), sealed quarters had twice as many intramammary infections (5.8%). The higher rate of intramammary infections in sealed quarters was mainly due to CNS (4.0% in sealed quarters, 2.5% in unsealed quarters). Based on random logistic regression models, the application of the external teat sealer was associated with increased odds of intramammary infections ($P < 0.001$), but not with clinical mastitis occurring in the first 100 days of lactation ($P = 0.165$). Further investigations are needed to characterize the dynamics of microbial populations under the external teat sealer.

Keywords: External teat sealer, polyurethane, udder health, mastitis, dry period, split-udder design

Introduction

The dry period of dairy cows is an important period concerning the development of mastitis with particular high susceptibility to intramammary infections (IMI) in the first and last days of the dry period [1]. IMI in the dry period often persist into lactation [2, 3]. Approximately one in two mastitis cases in early lactation is caused by a new infection during the dry period [2].

Not only multigravid cows, but also heifers are susceptible to prepartum IMI. 12.3-45.5% of udder quarters of heifers are infected at

calving [4]. IMI in late gestation and early lactation have a negative impact on the development of the mammary gland, udder health, milk yield and risk of culling [5].

Apart from the improvement of housing conditions, feeding and management, prepartum IMI and clinical mastitis in lactation may be reduced by antibiotic dry cow treatment (ADCT) of primigravid and multigravid animals [6, 7]. However, the limited duration of effect [8] requires additional measures like teat sealers which prevent mastitis pathogens from invading into the udder quarter. Internal teat sealers (ITS) reduce the rates of new infections and clinical mastitis during lactation [6, 9]. External teat sealers (ETS) based on tetrahydrofuran were proven to decrease somatic cell count (SCC) after calving [10], although their time of adherence is quite limited to an average of 3 or 4 days [10, 11].

Polyurethane was proven to be impermeable for microorganisms while being inhibitor-free and permeable for steam and oxygen [12]. It is widely used in wound care in human and veterinary medicine [13]. The objective of the present study was to investigate the effects of an ETS composed of plaster and polyurethane material on naturally occurring IMI and clinical mastitis in dairy cows and heifers in a German dairy herd.

Materials and Methods

Herd and animals:

The study was conducted on a commercial German dairy farm in Saxony-Anhalt between May and November 2012. The average herd size was approx. 1,100 lactating German Holstein black pied cows, housed in free stalls and producing an average of 9,500 kg milk per cow and lactation. The herd was chosen for this study because of its high heifer mastitis rate of 58% (% heifers of all heifers in one year, having more than 100,000 cells/ml in their first official milk recording) and its high dry period new infection rate of 26% (% dry cows of all dry cows in one year under 100,000 cells/ml in the last official milk recording before drying-off, having more than 100,000 cells/ml in their first official milk recording after calving). Only clinically healthy cows and heifers with four functional appearing teats without injury or traces of earlier violations were included in the study. Cows > 200,000 cells/ml milk in the last official milk recording before drying-off were excluded since they

may already have been infected in the previous lactation [14]. All cows received blanket ADCT with Benestermycin® (Boehringer Ingelheim, Ingelheim, Germany). Dry cows and pregnant heifers were on pasture and brought into a straw-bedded pen two weeks before expected calving.

The tested hypothesis was that sealed quarters will have a lower infection rate (5.5%) than untreated ones (11%) [15]. Based on a two-sided Chi-square test with type I error $\alpha = 0.05$ and type II error $\beta = 0.20$, a total of 390 quarter pairs were needed. If 10% of animals drop out of the trial post admission, approximately 215 cows were needed.

External teat sealer:

The prototype of the tested ETS was provided by Dr. Kenndoff GmbH & Co. KG (Hamburg, Germany). It consisted of commercially available adhesive tape (Beiersdorf AG, Hamburg, Germany) as carrier material with a round piece of polyurethane film in the middle (Fig. 1) which was positioned on the teat canal orifice. The carrier material was stuck around the teat barrel. All teats, sealed and control teats, were cleaned with a dry paper towel before application of the teat sealer.

Experimental design:

Late pregnant cows and heifers were examined for signs of impending parturition (especially udder oedema, leaking colostrum, vulva swelling and softening of the pelvic ligaments) every second or third day. In animals in which calving was expected within the next few days, the front left teat and the hind right teat were sealed by one of two authors. Front right and hind left teats were not treated (split-udder design). Teat sealers that still remained on the teat at calving were removed before the first milking at the latest.

Sampling procedure and data recording:

When study animals calved, it was recorded whether the teat sealers were still at the teats (TSC+) or had been lost before (TSC-). In the first (C1) and second (C2) week after calving, quarter foremilk samples of all udder quarters of each animal included in the study were taken aseptically by two trained researchers according to the German Veterinary Medical Society (GVMS) [16]. The first three streams of milk were discarded, then the teat end was scrubbed with a 70% alcohol-soaked single-use paper towel. Approximately 10 ml milk per quarter were milked aseptically into a sterile tube. C1 was taken on day 3 after calving at

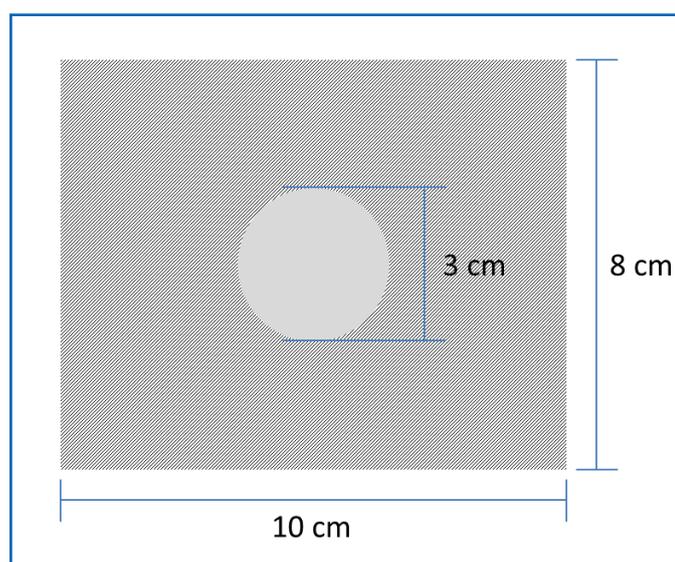


Figure 1: Sketch of the polyurethane-based external teat sealer. The dark area represents the adhesive tape, the light area represents the polyurethane material.

the earliest. Milk samples were transported at 5 °C to the microbiology laboratory of the University of Applied Sciences and Arts Hannover (Germany), where they were analysed immediately. Every clinical mastitis in the first 100 days of lactation was detected by redness, heat, swelling or changed secretion and recorded by the herd managers.

Laboratory analysis and definitions:

Milk samples were cultured according to NMC recommendations as published by GVMS [16]. Isolates were identified according to the recommendations of GVMS [17].

Environmental and opportunistic mastitis pathogens (coagulase-negative staphylococci (CNS), *Streptococcus (Sc.) uberis*, coliform bacteria and pseudomonads) were reported when five or more colonies had grown. According to NMC [18], a sample was defined as contaminated if more than two different types of colonies were detected. However, growth of *Staphylococcus (S.) aureus*, *Sc. agalactiae*, *Sc. dysgalactiae* and *Trueperella (T.) pyogenes* from contaminated samples was reported because these microorganisms are contagious mastitis pathogens which have their reservoir especially inside the mammary gland [17].

A quarter was considered infected if the same pathogen was detected in both C1 sample and C2 sample. If a quarter was only bacteriologically positive in either C1 or C2 sample or if different pathogens were cultured from C1 and C2 samples, the quarter was not considered infected. This was the case in 149 (76 quarters only positive in C1 and 73 quarters only positive in C2) and 14 quarters, respectively.

Statistical analysis:

The udder quarter was recognized as the statistical unit. The untreated opposed quarter served as a paired control. A quarter was matched with its contralateral teat. The quarter pair (front right and front left; hind right and hind left) was the unit of analysis. Infectious status after calving (I) and the occurrence of a clinical mastitis in the first 100 days of the following lactation (CM) were evaluated using two separate mixed model logistic regression analyses (SPSS Statistics 23, Armonk, USA), where number of lactation (first, second or higher lactation), treatment (ETS yes/no) and adherence of the teat sealer at calving (TSC+/TSC-) were included as fixed effects and the cow as a random effect. For the regression models the linear predictor was given by:

$$\text{Logit}(I, CM) = \text{number of lactation} + \text{treatment} + \text{adherence of teat sealer at calving} + \text{cow (random)} + e$$

Statistical significance was considered at $P < 0.05$.

Results

Of all cows that calved on the farm between May and November 2012, 223 animals, which fulfilled the inclusion criteria and which did not calve unexpectedly before being sealed, were included in the study (130 in first, 40 in second, 35 in third, 12 in fourth, 3 in sixth, 1 each in seventh, eighth and ninth gravidity). 13 of these animals did not finish the 100 days observation period, but all 223 enrolled animals finished the sampling period. Consequently, 892 udder quarters were sampled, of which 446 had been sealed. Primigravid animals calved on average 4.4 (0-21) days, multigravid animals 2.8 (0-9) days after application of the ETS. In primigravid animals, 26.6% of ETS (front left teat 26.9%, hind right teat 26.2%) and in multigravid animals 45.7% of ETS (front left teat 46.2%, hind right teat 45.2%) were still in place at calving. No negative impact on udder skin like allergic reaction or irritation was observed.

In sealed as well as in unsealed quarters, CNS were the most frequently detected microorganisms (4.0% and 2.5% of samples),

Table 1: Number and proportion of intramammary infections according to pathogen in sealed and unsealed udder quarters

	total number of quarters		unsealed quarters		sealed quarters					
	n = 892		n = 446		all sealed quarters		TSC+		TSC-	
	No.	%	No.	%	No.	%	No.	%	No.	%
CNS	29	3.3	11	2.5	18	4.0	5	3.2	13	4.5
primiparous	20	3.8	7	2.7	13	5.0	2	2.9	11	5.8
multiparous	9	2.4	4	2.2	5	2.7	3	3.5	2	2.0
<i>S. aureus</i>	6	0.7	2	0.4	4	0.9	1	0.6	3	1.0
<i>Sc. uberis</i>	3	0.3	0	0.0	3	0.7	1	0.6	2	0.7
<i>T. pyogenes</i>	1	0.1	0	0.0	1	0.2	0	0.0	1	0.3
Total infected	39	4.4	13	2.9	26	5.8	7	4.5	19	6.5

TSC+ = teat sealer still on the teat at calving

TSC- = teat sealer not on the teat at calving anymore

followed by *S. aureus* (0.9% and 0.4%), *Sc. uberis* (0.7% and 0.0%) and *T. pyogenes* (0.2% and 0.0%) (Table 1).

129 out of 892 quarters were culture-positive in C1 (14.5%) and 126 in C2 (14.1%). 76 of the 129 quarters, from which a pathogen was cultured in C1, were culture-negative in C2. According to definition, the rate of infected udder quarters after calving (same pathogen in C1 and C2) was 4.4% in total, 5.8% in sealed quarters (TSC+ 4.5%, TSC- 6.5%) and 2.9% in unsealed quarters. Different pathogens were cultured from C1 and C2 samples of 14 quarters, which were therefore not considered infected. In cows and heifers, 3.8% and 7.3% of sealed and 2.7% and 3.1% of unsealed quarters had an IMI, respectively. Quarters which had lost the ETS before calving had a higher risk of IMI than quarters on which the ETS remained until calving (in cows TSC+ 3.5% and TSC- 4.0%, in heifers TSC+ 5.8% and TSC- 7.9%). Significantly more sealed than unsealed udder quarters were bacteriologically positive ($P < 0.001$, OR = 3.571, CI = 2.306-5.531). Number of lactation and presence of the ETS at calving did not influence the rate of infected udder quarters significantly (Table 2).

The occurrence of clinical mastitis was evaluated for 210 of 223 animals. 13 animals were lost before their 100th day of lactation due to bad

milkability, low milk yield, mastitis, metabolic diseases, abomasal displacement or unknown reasons. Clinical mastitis in the first 100 days of lactation occurred in 9.4% of sealed quarters (TSC+ 8.3%, TSC- 9.9%) and in 8.3% of unsealed quarters. This difference was not statistically significant. In udder quarters of animals in third or higher lactation, significantly more clinical mastitis was recorded than in udder quarters of primiparous animals (Table 2).

Discussion

The period prior to calving is of particular susceptibility to IMI in cows and heifers [1, 19]. For the prevention of IMI, ITS and ETS are used. The aim of the present study was to investigate the effects of a polyurethane-based ETS applied a few days prior to calving on postpartum udder health of dairy cows and heifers.

All animals included into the trial were free of signs of clinical mastitis prior to calving. In addition, their last official milk recording in the previous lactation had to be $\leq 200,000$ cells/ml. This threshold is a value which is easily available to almost every farmer and which has also been used in earlier trials investigating the effect of ITS or ADCT on the new infection rate of dry cows [9, 15]. Although the official

Table 2: Logistic regression random effects models for risk of intramammary infection in the first two weeks of lactation and risk of clinical mastitis in the first 100 d of lactation

Variable	Intramammary infection			Clinical mastitis		
	OR	95% CI	P value	OR	95% CI	P value
Lactation number						
1	-	-	-	-	-	-
2	0.341	0.078-1.501	0.155	1.504	0.636-3.554	0.352
> 2	0.629	0.201-1.965	0.425	2.813	1.352-5.854	0.006
ETS						
no	-	-	-	-	-	-
yes	3.571	2.306-5.531	< 0.001	1.319	0.892-1.951	0.165
ETS at calving						
no (TSC-)	-	-	-	-	-	-
yes (TSC+)	0.841	0.343-2.063	0.705	0.753	0.397-1.428	0.385
Random effect of the animal		4.061-7.084	< 0.001		2.240-4.046	< 0.001

OR = Odds Ratio

CI = confidence interval

- = reference level

ETS = external teat sealer

TSC- = teat sealer not on the teat at calving anymore

TSC+ = teat sealer still on the teat at calving

milk recording cannot provide absolute sensitivity for the detection of mastitis at drying-off, it is an additional point of reference for excluding cows, which might be affected by mastitis, due to a high SCC.

Due to organisational reasons, teat sealing and sampling was not blinded. However, tasks in field and in laboratory were performed by separate staff, hence the influence on the results is considered to be small. Inclusion of only one farm may be another potential bias.

The duration of adherence was shorter than intended by the authors. 73.4% (primigravid animals) and 54.3% (multigravid animals) of ETS have been lost before calving. In earlier studies, the adherence of ETS was limited in time as well. The duration of adherence was 4 days (range 1 to 7 days) after treatment for DryFlex (DeLaval, Kansas City, USA) [11] and 2.95 ± 0.13 days for Stronghold (DeLaval, Kansas City, USA) [10]. Besides housing conditions, physiological changes of the udder morphology before calving, especially swelling of the teats (which is particularly strong in heifers), and leaking colostrum may be responsible for the limited time of adherence.

In the present study, a split-udder design was used to eliminate individual cow effects [20]. Still, not matching quarters with treatment groups randomly may possibly create a bias, eventually by teat condition impairments due to hyperkeratosis that tend to affect specific quarters because of e.g. positioning problems at milking. However, animals had to be free from clinical changes in the quarter morphology in order to participate in this study, and so the risk of this error was judged to be low.

The results show a doubled rate of IMI in sealed quarters (5.8%) compared to unsealed quarters (2.9%). Skin-associated pathogens prevailed. Environmental and contagious pathogens only played a secondary role in the peripartum period. Most IMI were caused by CNS, in sealed as well as in unsealed udder quarters. Only few *S. aureus*, *Sc. uberis* and *T. pyogenes* were isolated from infected udder quarters. The predominance of CNS corresponds to the results of studies concerning ITS, ADCT or combination treatments [7, 9, 15, 19] and to the high amount of heifers in this study, which are often infected with CNS [4]. SCC after calving was not taken into account in this study. Quarter level SCC measurement or a California Mastitis Test could have provided further information about potential cases of subclinical mastitis.

For ITS, a protective effect has been proven. ITS reduced four times the rate of new IMI postcalving in cows [15]. For heifers, the relative risk of IMI postcalving was 0.69 in sealed compared to unsealed quarters [7]. In contrast, in the present study, the rate of infected udder quarters was 5.8% in sealed quarters and 2.9% in unsealed quarters. Quarters treated with the ETS had significantly more IMI after calving. Hence, under the circumstances of this study, the polyurethane-based ETS had a negative effect on udder health.

However, the overall rate of IMI was low compared to other studies. This might be a consequence of the strict definition of IMI used in this study. Only quarters from which the same pathogen was isolated in C1 and C2 were declared to have an IMI.

Infections were mainly caused by CNS. Teat end and teat canal represent potential reservoirs for CNS [21]. Teat end and teat canal microbial loads under the ETS and their dynamics were not investigated in this study. It was shown that correlations between the bacterial counts on teat skin and in teat canals exist [22]. Contamination of the teat skin by microorganisms is a requirement for the colonisation of the teat canal, potentially followed by an invasion of pathogens into the teat cistern and the infection of the mammary gland [16]. The development of mastitis is facilitated by microbial colonisation of the teat canal [23] which in turn is decreased by the desiccation of the

teat canal after each milking. The evaporation of moisture is one of the primary defense mechanisms of the teat canal and limits microbial growth therein [24]. It might be that, due to the covering of the teat canal by the ETS, a humid milieu developed in teat canals. This would have improved the conditions for microbial colonisation of the teat canal and may have thereby caused the larger number of IMI in sealed udder quarters. Further investigations are required to analyse dynamics of microbial populations and multiplication conditions under the polyurethane-based ETS.

However, the fact that quarters which had lost the ETS before calving had a higher risk of IMI than quarters on which the ETS remained until calving questions this hypothesis. Maybe the ETS left sticky residues on the teat skin, which led to an increased contamination of the teat. Besides, disinfection of the teat skin prior to application of the ETS according to Lim et al. [10] and the incorporation of an antimicrobial substance in the polyurethane material could be considered in further trials.

Conclusions

Under these circumstances, the polyurethane-based ETS was not able to reduce the rate of IMI at calving in cows and heifers. Significantly more IMI, but not significantly more clinical mastitis cases in the first 100 days of lactation were recorded in sealed quarters. Insufficient disinfection before application and a possible development of a humid milieu under the ETS may be hypothesized as reasons and require further investigations.

Acknowledgements

We are grateful to Dr. Kenndoff GmbH & Co. KG for provision of the prototypes of the polyurethane-based ETS and to the dairy farmer for supporting this study. This project was funded by the German Federal Ministry of Education and Research, reference number 17107X10 (via AiF).

Conflict of interest

None declared.

References

1. Bradley AJ, Green MJ. The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. *Vet Clin North Am Food Anim Pract.* 2004; 20: 547-568.
2. Smith KL, Todhunter DA, Schoenberger PS. Environmental mastitis: cause, prevalence, prevention. *J Dairy Sci.* 1985; 68: 1531-1553.
3. Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ. Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. *J Dairy Sci.* 2002; 85: 2589-2599.
4. Fox LK. Prevalence, incidence and risk factors of heifer mastitis. *Vet Microbiol.* 2009; 134: 82-88.
5. Piepers S, de Vliegher S, de Kruif A, Opsomer G, Barkema HW. Impact of intramammary infections in dairy heifers on future udder health, milk production, and culling. *Vet Microbiol.* 2009; 134: 113-120.
6. Halasa T, Østerås O, Hogeveen H, van Werven T, Nielsen M. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *J Dairy Sci.* 2009; 92: 3134-3149.
7. Sampimon OC, de Vliegher S, Barkema HW, Sol J, Lam TJGM. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. *J Dairy Sci.* 2009; 92: 4395-4403.

8. Leslie K. Mastitis prevention strategies for the dry period. Proc. National Mastitis Council Regional Meeting. 1999; Waterloo, Ontario, Canada: 35-47.
9. Huxley JN, Green MJ, Green LE, Bradley AJ. Evaluation of the efficacy of an internal teat sealer during the dry period. *J Dairy Sci.* 2002; 85: 551-561.
10. Lim GH, Leslie KE, Kelton DF, Duffield TF, Timms LL, Dingwell RT. Adherence and efficacy of an external teat sealant to prevent new intramammary infections in the dry period. *J Dairy Sci.* 2007; 90: 1289-1300.
11. Lim GH, Kelton DF, Leslie KE, Timms LL, Church C, Dingwell RT. Herd management factors that affect duration and variation of adherence of an external teat sealant. *J Dairy Sci.* 2007; 90: 1301-1309.
12. Krömker V. Entwicklung eines bakterienundurchlässigen und heilungsfördernden Wund- und Zitzenkanalverschlusses für laktierende und trockenstehende Milchkühe auf der Basis eines innovativen Polyurethanwerkstoffes. Veröffentlichung der Ergebnisse von Forschungsvorhaben im BMBF-Programm. 2012. DOI: 10.2314/GBV:767768159.
13. Court MH, Bellenger CR. Comparison of adhesive polyurethane membrane and polypropylene sutures for closure of skin incisions in cats. *Vet Surg.* 1989; 18: 211-215.
14. Pantoja JC, Hulland C, Ruegg PL. Dynamics of somatic cell counts and intramammary infections across the dry period. *Prev Vet Med.* 2009; 90: 43-54.
15. Krömker V, Pfannenschmidt F, Friedrich J. Neuinfektionsrate der Milchdrüsen von Milchkühen in der Trockenperiode nach Anwendung eines internen Zitzenversieglers zum Trockenstellen. *Berl Münch tierärztl Wochenschr.* 2010; 123: 215-220.
16. German Veterinary Association (GVA). Guidelines for antiseptic milk sampling and guidelines to isolate and identify mastitis pathogens. GVA. 2009; Gießen, Germany.
17. German Veterinary Association (GVA). Guidelines for bovine mastitis control in dairy herds. GVA. 2012; Gießen, Germany.
18. National Mastitis Council (NMC). Laboratory Handbook on Bovine Mastitis. Revised edition. NMC. 1999; Madison, WI, USA.
19. Bradley AJ, Breen JE, Payne B, Green MJ. A comparison of broad-spectrum and narrow-spectrum dry cow therapy used alone and in combination with a teat sealant. *J Dairy Sci.* 2011; 94: 692-704.
20. Barkema HW, Schukken YH, Lam TJGM, Galligan DT, Beiboer ML, Brand A. Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. *J Dairy Sci.* 1997; 80: 1592-1599.
21. Taponen S, Björkroth J, Pyörälä S. Coagulase-negative staphylococci isolated from bovine extramammary sites and intramammary infections in a single dairy herd. *J Dairy Res.* 2008; 75: 422-429.
22. Paduch JH, Krömker V. Colonization of the teat skin and the teat canal by mastitis pathogens in dairy cattle. *Tierärztl Prax.* 2011; 39: (G) 71-76.
23. Zecconi A, Hamann J, Bronzo V, Ruffo G. Machine-induced teat tissue reactions and infection risk in a dairy herd free from contagious mastitis pathogens. *J Dairy Res.* 1992; 59: 265-271.
24. Williams DM, Mein GA. The role of machine milking in the invasion of mastitis organisms and implications for maintaining low infection rates. *Kiel Milchwirtschaftl Forschungsber.* 1985; 37: 415-425.