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The Effect of Sour Milk as a Postmilking Teat Dip for Mastitis Prevention in a Dairy Herd

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Koskinen, E., M. Rantala and H. Saloniemi: The effect of sour milk as a postmilking teat dip on somatic cell count and bacterial growth in a dairy herd. Acta vet. scand. 1996, 37, 427-432. – In a preliminary in vitro study, the growth of *Staphylococcus aureus* was totally inhibited during incubation for 24 h at 35 °C–37 °C in a solution of cooked commercial milk with 1% of uncooked commercial sour milk ("A piimä"). In a subsequent clinical trial, "A piimä" sour milk with 5% glycerol was used as a postmilking teat dip from February to June. Quarterly milk samples were drawn once a month aseptically from 133 cows. Percentages of pathogen positive samples and somatic cell count (SCC) from teats dipped with the sour milk were compared with those dipped with a commercial iodine teat dip and those of undipped controls. During March–June there were fewer isolations of *S. aureus* (2.09%) and coagulase-negative staphylococci (2.52%) in the sour-milk group than in the control group (3.09% and 4.07%, respectively). In iodine group, there were fewer isolations of *S. aureus* (0.83%) but more isolations of coagulase-negative staphylococci (5.26%) than in the control group. During the study period, the percentages of bacterial isolates did not differ statistically significantly between treatments, $p = 0.291$. The percentage of quarters with a SCC over 125,000 at the end of the study was one third lower in the sour-milk group than in the control group (16.67% and 26.23% respectively) but the difference was not statistically significant ($p = 0.074$). The results indicate that a sour-milk teat-dip preparation can inhibit new intra mammary infections (IMI).

Lactobacillus acidophilus; SCC; IMI; bovine.

Introduction

Numerous post-dipping studies have been presented where different formulations are described to reduce the rate of bovine mastitis *(Pankey* 1992). However, one limiting factor in the use of many formulations is the risk of residues in milk. *Woodward et al.* (1987) have reported that approximately 25% of the isolates of normal flora recovered from the teat ends of healthy heifers inhibit in vitro the growth of Gram-positive and Gram-negative udder pathogens. They suggested that colonization of teat ends with normal flora inhibiting pathogens in vitro may increase nonspecific resistance to mastitis. *Lactobacillus acidophilus* has been reported to exert antagonistic actions on growth of *Staphylococcus aureus* among other pathogens when grown with each in associative cultures *(Gilliland & Speck* 1977). We could find no published data on the effect of dipping the teats of milking cows with solutions containing lactic acid bacteria to inhibit new intra mammary infections (IMI).

In the 2 studies reported here, we first examined the ability of one lactobacillus culture and 5

sour milk products (cooked and uncooked) to inhibit the in vitro growth of *Staphylococcus aureus,* an important cause of acute and chronic bovine mastitis. In a subsequent clinical trial we used the most effective sour milk product from the in vitro study as a postmilking teat dip. Percentages of bacteria positive milk samples and somatic cell count (SCC) in quarter milk samples from these quarters were compared with those from teats dipped with a commercial iodine teat dip, and from undipped controls.

Materials and methods

The *S. aureus* strains used in the in vitro study were isolated from clinical cases of mastitis at Kemijärvi region. The growth of inocula of 10–100 cells of *S. aureus* (0.1 ml of a 10^{-7} dilution of a *S. aureus* bouillon culture incubated for 24 h at 37 °C) was examined in tubes containing 9.9 ml of cooked, commercial milk. To these tubes were added 0.1 ml of one of the following sour milk products: uncooked or cooked commercial Finnish sour milk "A piimä", or uncooked or cooked commercial Finnish sour milk "Gefilus", or commercial Finnish buttermilk "Kirnupiimä", or a *Lactobacillus spp.* culture made of lyophilized bacteria (Laboratoires Lyocentre, France) incubated for 24 h at 39 °C in cooked milk. The trial was repeated once with "A piimä" sour milk. "A piimä" sour milk included the following lactic acid bacteria: *L. acidophilus, Lactococcus lactis* ssp. *lactis* and *cremoris, Lactococcus lactis* ssp. *lactis* var. *diacetylactis,* and *Leuconostoc mesenteroides* ssp. *cremoris.* "Gefilus" included the same bacteria as "A piimä" and *Lactobacillus GG.* Tubes were incubated for 24 h at 35 °C–37 °C and then sub-cultured on bovine blood agar plates and Baird Parker agar plates.

After the in vitro study, a clinical trial was conducted at the Agricultural Research Centre in Finland. The animals were kept in 3 cowhouses with, on average, 60 cows in each. The animals were of ayshire breed, they were on average 3.8 years (± 1.5 SD) old and they had been lactating on average for 3.2 months (± 2.1 SD) at the beginning of the study. Initial quarter milk samples were collected in February 1993. Based on these samples the animals were put to groups on the basis of general udder health, in terms of the highest SCC in any quarter. SCC limits were 125,000, 250,000 and 1,500,000. The treatment subject in the study was udder half. Within the cow groups, udder halves were assigned at random to: no treatment, dipping with "A piimä" sour milk, including 5% of glycerol to prevent drying of the teat skin, or dipping with a commercial iodine dip (Tehotippi, Orion-Farmos OY, Finland: iodine concentration after dilution for use is 0.15%). The experimental design was incomplete block design with 2 treatment subjects (left and right udder halves) and 3 treatments (SAS Proc plan, SAS Institute Inc., Cary, NC, USA). Because udder halves were assigned at random to treatment groups, the distribution of age, lactation phase, cowhouse, management and related factors were similar in all treatment groups. Dipping was started 5 or 6 days after the initial milk samples had been collected. Two to 5 centimeters of a teat were dipped. Quarter milk samples were collected aseptically before milking in the morning, once a month, from March to June. After milking, the samples were put in a refrigerator. SCC was measured within 6 h after milking. Cells were counted using an automatic cell counter (Fossomatic). Before counting of cells, milk samples were warmed to 40 °C. The rest of the milk sample was frozen at –20 °C and sent for bacterial examination. Only pathogenic bacteria were examined. *S. aureus,* coagulase-negative staphylococcus (CNS), *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Enterococcus faecalis* and *faecium, Actinomyces pyogenes, Corynebacterium*

bovis, Escherichia coli and *Klebsiella* were identified according to *Honkanen-Buzalski & Seuna* (1995). One or more colonies of bacteria on an agar plate was considered as an indication of bacteria positive sample in this study. There were 12.1% of milk samples with less than 5 colonies on agar plate of all bacteria positive milk samples. Before the beginning of the study, other changes had been made to milking practices; e.g. milkers had began to use disposable milking cloths. The changes were similar in all treatment groups.

McNemar's test for correlated proportions was used to determine the significance of change in percentages of bacterial isolations from February to March. The chi-squared test was used to determine whether treatments had significant effects on percentages of bacterial isolations overall during treatment, or on percentages of quarters with SCC>125,000 at the end of the study.

Results

In vitro study

S. aureus growth was totally inhibited by one per cent of uncooked commercial sour milk "A piimä" during incubation at 35 °C–37 °C for 24 h in cooked commercial milk. The number of S. aureus was 10^2–10^3 per ml after incubation with one per cent of sour milk "Gefilus", *Lactobacillus* culture or the buttermilk "Kirnupiimä". The number of *S. aureus* was 10^7 per ml after incubation with one per cent of cooked sour milks "A piimä" or "Gefilus", or in the control tube (Table 1).

Clinical study

During the study period 11 cases (6 *S. aureus,* 4 *Enterococcus faecium* and *faecalis* and 1 coagulase-negative staphylococcus) of new clinical mastitis were recognized (a positive bacterial diagnosis and SCC over 300,000 per ml of milk). There were 3 cases, 2 cases and 1 case of clinical *S. aureus* IMI during the study in the control, "A piimä" sour milk and iodine groups, respectively.

The decreases in percentages of bacteria-positive milk samples from February to March were statistically significant in relation to all treatments, ($p < 0.001$). The decreases did not differ significantly between treatments (Table 2).

During the treatment period, the smallest proportion of *S. aureus* positive milk samples was found in milk samples from iodine-dipped quarters. The percentage of CNS positive samples was lowest in the "A piimä" sour milk group. Both dipping groups had more *Entero-*

Table 1. Growth of *Staphylococcus aureus* inoculated in cooked commercial milk to which had been added various sour milk products.

Source	Bovine blood agar cells/ml	Baird-Parker agar cells/ml
Control	10×10^7	10×10^7
Sour milk "A piimä"	0	0
Cooked "A piimä"	9×10^7	5×10^7
Sour milk "Gefilus"	3.7×10^2	3.3×10^2
Cooked "Gefilus"	6×10^7	6×10^7
Lactobacillus acidophilus culture	2×10^2	7×10^2
Buttermilk "Kirnupiimä"	2×10^2	1.5×10^2

Table 2. Percentage of bacteria positive milk samples.

Dip group	Number of quarters	Start of dipping (Feb)	March	April	May	June
Control	180	18.3	6.7***	13.3	11.1	7.8
Sour milk + 5% glycerol	170	14.1	1.8***	12.2	10.6	7.7
Iodine	182	15.4	6.0***	13.6	12.1	9.9

Levels of significance in relation to percentages at start of dipping in February: *** = ($p<0.001$).

coccus faecalis and *faecium* isolations than the control quarters. Percentages of bacterial isolates overall did not differ significantly between treatments ($p = 0.291$) (Table 3).

In cows with SCC in initial milk samples less than 125,000, the percentage of quarters with SCC over 125,000 at the end of study was about one third lower in the "A piimä" sour milk group than in the control group, (16.67% and 26.23%, respectively, $p = 0.074$) (Table 4).

Discussion

In our in vitro study, sour milk containing several species of lactic acid bacteria was effective in inhibiting *S. aureus* growth. We found differences between commercial sour milk products in relation to inhibition of *S. aureus* growth. According to *Woodward et al.* 1987, ability to inhibit is probably not characteristic of a genus or species but of specific strains of bacteria. The efficacy of any sour milk product should therefore be evaluated in vitro before it is used in practice.

In our clinical study, milk samples were warmed for some minutes to 40 °C before counting of cells. Warming might have influenced the results of bacterial examination. However, warming was similar for all samples. The percentages of bacteria positive milk samples decreased in all treatment groups one month after the beginning of the study. It is generally accepted that improvement in udder hygiene greatly decrease numbers of bacteria on teat ends *(Pankey* 1992). It is probable that milking hygiene was better than usual among milkers for some weeks after the beginning of the study.

Because there were only 6 new cases of clinical S. aureus IMI with SCC>300,000 during the study, no comparisons between the groups could be analyzed statistically.

In the study reported here, the percentages of *S. aureus* positive milk samples were lowest in the iodine group. The percentages of *S. aureus* or CNS positive milk samples in the sour milk group were lower than those in the control group. This finding is comparable to the findings of *Oliver & Mitchell* (1985). In their study, *L. acidophilus* teat dip preparation was effective against *S. aureus* infections in a commercial dairy herd. However, exact figures are not given in their report.

The decrease in percentage of healthy quarters (low SCC) after May could have been a seasonal effect. Temperature in cowhouses rise towards summer, causing stress and leading to high SCC *(Saloniemi* 1980). In summer, proper udder hygiene in milking management is apparently more difficult, when cows are on pasture and milked at summer milking stations. It is probable that infection pressure increased in

Table 3. Percentages of bacteria positive milk samples during March–June.

Bacterial spp	Treatment group: Control (712)[1]	Sour milk + glycerol (5%) (674)	Iodine (722)
Staphylococcus aureus	3.09	2.09	0.83
Coagulase-negative staphylococci	4.07	2.52	5.26
Streptococcus dysgalactiae	0.56	0.59	0.69
Streptococcus uberis	0.14	0	0.97
Enterococcus faecium and *faecalis*	1.40	2.23	2.22
Other pathogens[2]	0.42	0.59	0.42
Total	9.69	8.01	10.39

[1] Number of quarters.
[2] Other streptococci and yeast.

Table 4. Percentage of udder quarters with somatic cell count >125.000 cells / ml of milk in cows with initial somatic cell count ≤125.000 cells / ml of milk in all quarters.

	Number of quarters	Start of dipping (Feb)	March	April	May	June
Control	122	0	5.74	5.08	6.56	26.23
Sour milk	114	0	1.75	6.48	5.31	16.67
Iodine	124	0	3.20	4.07	8.00	21.60

group seemed best protected against high SCC levels. Differences between treatments might have been more visible if the study had been continued through the summer. The study reported here was probably stopped too early.

The sour milk dip used in the study reported here included 5% of glycerol. We did not examine the possible health effect of glycerol on teats. Also the mechanism of how the sour milk affects the teat and udder health was not studied.

Sour milk does not cause a residue problem. Other advantages of sour milk as a post milking teat dip could be that it is easily available as fresh cultures in ordinary shops in many countries, and is cheap.

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Sammanfattning

Inverkan av spendoppning med sur mjölk på uppkomsten av nya juverinflammationer.

I den första studien tillsattes 1% okokt sur mjölk "A piimä" till kokt mjölk. Blandningen förhindrade *Staphylococcus aureus* tillväxt in vitro. I den följande kliniska studien användes "A piimä" med 5% glycerol som ett spendoppningsmedel efter mjölkning från och med februari till och med juni. Juverfjärdedelsprov togs en gång i månaden från 133 kor. Bakteriefynd och SCC i mjölkprov från juverfjärdedelar som doppats med sur mjölk jämfördes med dem som doppats med spendoppningsmedel innehållande jod och med dem som inte doppats. Färre *S aureus* (2.09%) och CNS (2.52%) påvisades i "surmjölksgruppen" än i kontrollgruppen (3.09% och 4.07%, respektive). I jodgruppen förekom färre *S aureus* positiva mjölkprov (0.83%) men flera CNS positiva mjölkprov (5.26%) än i kontrollgruppen. Skillnaderna var inte statistiskt signifikanta (p = 0.291). Procenten juverfjärdedelar med somatiska celler över 125,000 i slutet av studien var en tredjedel mindre i "surmjölksgruppen" än i kontrollgruppen (16.67% och 26.23%, p = 0.074). Resultaten indikerar att surmjölk kan minska uppkomsten av nya juverinflammationer.

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