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University of Veterinary Medicine
Research Institute of Veterinary Medicine
Hlinkova 1/A
040 01 Košice
Slovak Republic

MICROBIOLOGICAL LOAD OF THE ENVIRONMENT AND DISINFECTION IN THE STABLES OF DAIRY COWS

Vasiľ, M., Gréserová, G., Venglovský, J., Plachá, I., Sasáková, N., Pačajová, Z., Dostál, A.*

Research Institute of Veterinary Medicine of the UVM,
Hlinkova 1/A, 040 01 Košice, Slovak Republic, vasil@vuvm.sk
Ministry of Agriculture Slovak Republic

ABSTRACT

The activity of four disinfectant solutions used for the post-milking teat dip was observed throughout 40 weeks on a dairy farm with an average number of 395 cows. The animals were housed in two production stables in free boxes and milked in the Boumatic 10 milking parlour. The herd was subjected to complete bacteriological and clinical examination and to the NK test at weekly intervals. The effects of the disinfectants observed in the experimental animals were compared to a group of controls. The activity of the preparation was tested in four experimental groups of dairy cows:
In Groups 1 (61 animals) and 2 (60 animals) 5% Nolvasan TD and Jodonal M were used for post milking teat dip, respectively. In Group 3 (61 animals) Benzitrin at a dilution of 1:4, in Group 4 (61 animals) Bou Matic Hexi and in the control group a sterile solution stained with red foodstuff colour 195 were applied. By the method of post-milking teat disinfecton a 60.4% reduction of the occurrence of new intramammary infections (IMI) was achieved in Group 1 (Nolvasan TD) as compared to the controls whereas this reduction in the groups 2, 3 and 4 presented 50.0%, 42.6% and 31.4%, respectively. Among all infections of the mammary gland determined throughout the experiment major mammary gland pathogens were prevailingly isolated from 192 dairy cows - 43.4% (Streptococcus agalactiae 5.2%, Str. Dysgalactiae 3.8%, Beta-haemolytic Streptococci 16.1% and Staphylococcus aureus 18.3%), environmental pathogens were found in 92 dairy cows - 20.8% (Str. uberis 15.8%, Escherichia coli 5.0%) and minor pathogenic bacteria (coagulase-negative Staphylococci) were proved in 158 dairy cows - 35.8% .

INTRODUCTION

Contamination of the housing facilities of dairy cows with pathogenic microorganisms is frequently reflected in the morbidity rate of the animals. Recently increased occurrence of environmental pathogen-induced mammary infections has been stated. Frequent and thorough cleaning of the stables, regular disinfection as well as strict keeping to the hygienic milking programme are an important means of decreasing the exposition of the udder of dairy cows to environmental microorganisms. Disinfection is generally one of the most important ways both of disease prevention and of sanitation of the environment (Sasáková et al., 1999; Plachá et al. 2001; Ondrašovič et al., 1998; Venglovský et al., 1996). Post-milking teat dip into an effective disinfectant carried out within one minute after milking presents an important element of the hygienic milking programme, however, the basic aim of the post-milking teat dip, i.e., prevention of new infections or some of the forms of mastitis, is not always kept in mind and thus shortcomings like an inadequately chosen disinfectant, insufficient concentration of the effective substance or delayed application of the disinfectant after milking are frequently seen (Vasiľ, 2000; Pačajová, 1990). Post-milking teat disinfection is one of the most effective means to prevent infections of the mammary gland since it kills 85% of the bacteria contaminating the skin of the teat either during preparation of the milking process or during milking itself. After milking the teat duct closes very slowly, as long as 2 hours. Milk drops that remain on the

tip of the teat are drawn into the teat duct by capillary elevation, carrying with them also the bacteria from the tip of the teat. It is disinfection of the teat after conclusion of the milking process that reduces this penetration of bacteria into the teat duct by as much as 90%. The cow should therefore not lay down for two hours after milking and it should be fed after milking (Škarda et al., 1989). Disinfection of the teat tips after milking is an important part of the hygienic regime used for prevention and control of staphylococcal infections, infections caused by S. agalactiae, S. dysgalactiae and environmental pathogenic bacteria. The occurrence of new infections induced by these agents is then decreased by as much as 50%.

It was the aim of this research to verify the activity of 4 disinfectants intended for post-milking teat dip in order to reduce the occurrence of new intramammary infections of the mammary gland as well as the average number of all infections per dairy cow.

MATERIALS AND METHODS

Examinatios were carried out on a farm with an average number of 395 dairy cows. The animals were housed in two production stables in free boxes and milked in the Boumatic 10 milking parlour . As delivery rooms two reconstructed K98 stables with conventional technologies were used. In the course of 3 months prior to starting the experiment the hygienic conditions in the stables were stabilized, biotechnical control of the equipment was carried out and the correctness of conducting the single elements of the hygienic milking programme evaluated. At the same time all animals secreting pathogenic microorganisms of the mammary gland were treated with antibiotics according to Vasil' (2000). For treatment mainly the intramammary preparation Cefa Lak (Fort Dodge, USA) was used. Prior to starting the observation complex examination of the dairy cows in the herd was carried out:

* clinical examination of the udder (IDF, 1987), and
* bacteriological examination of secretion samples from the mammary gland (IDF, 1981).

On the basis of the results obtained by the first complex examination the dairy cows were allotted to 5 groups (according to the box sections with a capacity of 60 animals each) as follows:

- Group 1 - average 61 dairy cows - after milking the teats were treated by dipping into 0.5% Nolvasan Teat Dip
- Group 2 - average 60 dairy cows - after milking the teats were treated by dipping into 25% Jodonal M
- Group 3 - average 61 dairy cows - after milking the teats were treated by dipping into Benzitrin conc. sol. a. u. v. at a dilution of 1:4
- Group 4 - average 61 dairy cows - after milking the teats were treated by dipping into Bou - Matic Hexi Dip
- Group 5 - average 60 dairy cows which served as controls - after milking the teats were treated by dipping into sterile distilled water stained with red foodstuff colour 195.

Nolvasan TD containing 5% chlorhexidin acetate, 39% glycerin, inert ingredients g.s. was used as a 5% solution.

Jodonal M containing 18.6% iodine, corrigentia in g.s. in 1 kg, was used as a 20% solution.

Benzitrin conc. sol. a.u.v. containing 2 g benzyldodecyldimethylamoniichloridum, 50 mg methylrosanillinii chloridum, 1 g polymethylsiloxan, 10 g alcoholum polyvinylicum,

corrigentia in g.s. in 1 l was used at a dilution of 1:4.
Bou-Matic Hexi Dip. lig. a.u.v. containing 5.0 g chlorhexidin digluconas ad 1000 ml was used undiluted.

The effectivity of the disinfective solutions was observed for 40 weeks at weekly intervals by means of the abovementioned complex examination of the health state of the mammary gland (by clinical examination of the udder and bacteriological examination of the milk). Effectivity of the disinfective solutions was determined on the basis of the number of persisting or new infections in the groups under examination. The diagnosed infections were classified as follows:

Persisting infection of the mammary gland - state of a mammary gland in the secretion of which pathogens similar to those obtained in the previous examination were found.

New infection of the mammary gland - state of a mammary gland from the secretion of which other pathogens were isolated, i.e., at subsequent examination pathogens were isolated from the originally negative mammary gland.

Frequency of the occurrence of new infections per dairy cow - share of the sum of new infections of the mammary gland throughout the period of examination and of the average number of dairy cows during that period.

Reduction of frequency of the occurrence of new intramammary infections - percentual expression of the decreased frequency of occurrence of new intramammary infections per dairy cow for the period of observation as contrasted to the control group.

RESULTS

From Table 1 it follows that in contrast to the controls in which alltogether 151 infections were determined, the smallest number of new infectons (44) was observed in the Nolvasan TD-treated group of dairy cows. In comparison with this group other groups revealed a substantially higher occurrence of infections that in the Bou Matic Hexi-treated group were even more than 2fold. The average number of infections per week observed in the single groups corresponds to the above mentioned state. The effectivity of the preparations under examination has been proved by the average value of the occurrence of new infections per one animal. In contrast to the controls a reduction of new infections by 50.0% was observed in the groups of dairy cows treated with Jodonal M and Nolvasan TD, whereas in the other two groups this value was below 50.0%. It should be stated that the average values of the occurrence of new intramammary infections per animal in all groups examined including the control group, proved a relatively good zoohygienic standard of the farm under observation.

From Table 2 it follows that environmental and minor pathogens were the overall dominant pathogens (56.6%) that caused mamary gland infections (Streptococcus uberis, Escherichia coli and coagulase-negative Staphylococci caused 15.8%, 5.0% and 35.8% of the infections, respectively). Major contagious bacteria caused 43.4% of the infections, with Staphylococcus aureus, beta-haemolytical Streptococci, Streptococcus agalactiae and Str. dysgalactiae found in 18.3%, 16.1%, 5.2% and 3.8% of the infections, respectively. In the groups treated with disinfecting solutions the following contagious pathogens were prevailingly isolated: Streptococcus agalactiae in the Jodonal M treated group (21.7%), Staphylococcus aureus in the Bou Matic Hexi - treated group (21.0%), Streptococcus dysgalactiae in the Jodonal M and Benzitrin-treated group (17.6%) and beta-haemolytical Streptococci in the Bou-Matic Hexi-treated group (21.1%).

Of the environmental pathogens, Streptococcus uberis and Escherichia coli were prevailingly isolated in the Bou-Matic Hexi and the Jodonal M -treated groups, respectively (24.3% vs. 17.3%). Coagulase-negative Staphylococci were prevailingly isolated in the Bou-Matic Hexi-treated group (27.1%).

DISCUSSION

The results presented in this study are similar to those published by Boddie et al. (1993) who achieved a reduction of Staphylococcus aureus and Streptococcus agalactiae-caused IMI by 65% and 33.3%, respectively, when using a 0.175% solution of a iodine preparation (4-6 ppm of free iodine).

Table 1 *Occurrence of persistent and new infections and reduction of the incidence of new infections in groups of dairy cows treated with Nolvasan Teat Dip, Jodonal M, Benzitrin and Bou-Matic Hexi during a period of 40 weeks in comparison with the controls*

Week of examination	Group of dairy cows treated with the disinfectant									
	Nolvasan TD		Jodonal M		Benzitrin		Bou-Matic Hexi		Control	
	P	N	P	N	P	N	P	N	P	A
Number of infections within 40 weeks	28	16	49	27	49	31	54	37	97	54
Infections Total	44		76		80		91		151	
Average number of infections per week and group	1.10		1.95		2.00		2.28		3.78	
Average number of cows	61		60		61		61		60	
New infections per 1 dairy cow	0.26		0.45		0.51		0.61		0.9	
Reduction of new infections in %	60.4		50.0		42.6		31.5		0.00	

P - *number of persistent infections*; N - *number of new infections*

Drechsler et al. (1993) tested two formulations of a 0.5% chlorhexidin digluconate germicide with 0.5 % glycerin under the conditions of experimental challenge; the highest efficacy reached was 72.8 % against Staphylococcus aureus and 44,4% against Streptococcus agalatiae. Boddie et al. (1997) used experimental challenge to test two disinfecting solutions for 5 weeks. The use of 0.5% chlorhexidin digluconate containing 4% glycerin reduced the occurrence of Staphylococcus aureus and Streptococcus agalactiae-induced IMI by 73.2% and 53.9%, respectively. Using 1% iodine (12 ppm of free iodine) containing 10% glycerin these authors achieved a reduction of new IMI caused by the above two pathogens by 75.6% and 53.5%, respectively. The latter authors concluded that in comparison to the controls the occurrence of the clinical forms of mastitis was reduced in the experimental animals.

It is obvious that besides perfect cleaning of the udder pre-milking teat disinfection which can contribute to a pronounced reduction of the occurrence of new IMI and

Table 2 Distribution of bacterial agents isolated from the milk of dairy cows in groups treated with Nolvasan Teat Dip, Jodonal M, Benzitrin and Bou-Matic Hexi as well as in the control group during a period of 40 weeks

Infectious agent	Group of dairy cows treated with the disinfectant										Total	
	Nolvasan TD		Jodonal M		Benzitrín		Bou-Matic Hexi		Control			
	P	%	P	%	P	%	P	%	P	%	P	%
Streptococcus agalactiae	2	8.7	5	21.7	4	17.4	3	13.0	9	39.1	23	5.2
Streptococcus uberis	5	7.1	11	15.7	11	15.7	17	24.3	26	37.1	70	15.8
Streptococcus dysgalactiae	2	11.8	3	17.8	3	17.6	2	11.8	7	41.2	17	3.8
„β-haemolytical“ Streptococci	7	9.9	11	15.5	14	19.7	15	21.1	24	33.8	71	16.1
Staphylococcus aureus	8	9.9	12	14.8	14	17.3	17	21.0	30	37.0	81	18.3
Coag. – negative Staphylococci	17	10.8	28	17.8	29	18.4	35	21.2	49	31.0	158	35.8
Escherichia coli	3	13.6	6	27.3	5	22.7	2	9.1	6	27.3	22	5.0
Total	44	10.0	76	17.2	80	18.1	91	20.6	151	34.2	442	100.0

subsequent inflammatory changes in the mammary gland presents an important part of the hygienic programme of milking (Lenhardt a kol., 1999).

Oliver et al. (1994) observed the effectivity of 0.35% chlorhexidin teat dip as a pre-milking teat disinfectant in the reduction of naturally occurring new IMI. Predipping was compared with a negative control using a split-udder experimental design. All teats were dipped after milking with sane 0.35% chlorhexidine teat dip. Most new major pathogen-induced intramammary infections were caused by Streptococcus species, primarily S. uberis and S. aquinus, and by Gram-negative bacteria, primarily Escherichia coli. The percentage of quarters newly infected by major mastitis pathogens was by 30.6% lower in mammary glands with teats pre- and post-dipped in chorhexidine than in mammary gland with teats post-dipped only. New infections by coagulase-negative Staphylococus species were significantly decreased in mammary glands with teats pre- and post-dipped than in mammary glands with teats post-dipped only. When all mastitis pathogens were combined, the percentage of quarters newly infected by major and minor pathogens was significantly lower in the pre- and post-dipped group than in the post-dipped-only group.

In connection with the prevention of mastitis in dairy cows it is important to realize that as many as 80% of the infections are caused by Str. agalactiae, Str. dysgalactiae, Str. uberis, Streptococci of the serological groups C, G and L, Staphylococcus aureus and coagulase-negative Staphylococci; this has also been confirmed in our investigation.

The fact that mammary gland infections are secondary to a decrease in the defense mechanisms in consequence of the influence of a whole series of internal and external factors speaks well for disinfection of the tips of teats prior to and after milking. Almost all mammary gland infections emerge via the teat duct when the mechanisms active in the upper part of the duct and preventing bacteria from penetrating the teat are overcome by the latter.

The frequency of occurrence of new intramammary infections per one dairy animal during lactation is rather low (1.4) which was confirmed in this research in which we achieved an even lower value (0.9 - see Table 1) in the control group. The long duration of IMI (up to 7 months) is a well known fact of the utmost disadvantage.

Fortunately the defense ability of the mammary gland is not so very low since as much as 39.0%, 40.0% and 55.0% of new IMI caused by Streptococcus sp., Staphylococcus sp. and coliforms, respectively, are spontaneously eliminated.

Concluding it can be stated that, when compared with the controls, post-milking teat dip into a disinfectant solution was effective in reducing both the ocurrence of new IMI and the average number of all infections in a group per dairy cow and week. It could be confirmed that the method of post-milking teat dip into an effective disinfectant solution was an important element in the hygienic programme of milking and in the prevention of mastitis in dairy husbandries as such.

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