Udder preparation and its effect on udder cleanlenees and milk quality

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Abstract

Total bacterial count was performed in eighty milk samples and eighty udder swabs collected from 20 cows, to compare the effect of treating udders with wet towel washing (55-60C^0), followed by paper towel drying; three concentrations of Iodophre and Hibitane followed by paper towel drying and two concentrations of potassium permanganate followed by paper towel drying with non-treated udders .Results showed that Log10 of CFU/ swab of untreated udders was significantly ($p\leq 0.05$) differ from those swab samples when udder was treated with wet towel washing (55-60C^0), followed by paper towel drying. By using iodophor, there was in asignificant ($p\leq 0.05$) reduction in the Log10 of CFU/ swab after udder treatment with all used concentrations of iodophor, and there was also proportional reduction in the Log10 CFU/ swab with each increase in the Iodophor concentration (0.1:100, 0.5:100 and 1:100) respectively. The same picture was gained by using Hibitane at concentrations of 0.25%, 0.5% and 1% as Iodophre. Potassium permanganate treatments with its two concentrations showed significant differences in Log10 /swab of udder before and after treatments and between its two concentrations 0.5:1000 and 1:1000.Total bacterial count of milk samples were a mirror of swab samples. From results it is evident that chemical disinfectants or hot water were effective in reducing udder contamination before milking.

Introduction

In healthy cows free from infection, milk emerging from the udder is essentially sterile, but it may contain commensal bacteria associated with the udder, since significant numbers of organisms may be found in milk taken even in a manner that prevents microbial contamination (i.e. aseptically) from the udders of apparently healthy cows. Limond and Griffiths (1) obtained counts of about 100 cfu/ml in aseptically drawn milk. The external surface of the udder is a prime source of microbial contamination of milk. Bedding materials, mud, faces, soil and other matter all readily stick to skin and are a rich source of microorganisms. Even after washing with water, the microbial count on teat surfaces can be high (2) and the count in milk from washed udders may only be about 1 log cycle lower than from those that were unwashed (3). Similar low-level reductions in total microbial count and coliform counts on both the udder surface and in milk were observed even after the use of disinfectants to treat teats (4,5). Sanaa et al. (6) showed that poor cleanliness of cows, inadequate lighting of milking parlors and barns (which may be an indication of neglect of milking hygiene) and incorrect disinfection of towels used to dry the udder significantly increased the likelihood of contamination.It has been suggested that bedding affords the greatest contribution to external udder contamination (7). There was a reduction in bacterial levels on teats when cows were on pasture and this was reflected in lower bacterial counts in milk during this period. The bacterial count of all types of bedding was about 5 _ 109 cfu/g whereas that on pasture was approximately 8 _ 107 cfu/g (8). The dominant microflora on the teats of cows housed in barns were micrococci (9) but it has also been estimated that 90% of the spores found in raw milk come from this source (10).Although bedding can be a significant source of udder contamination, arguably fecal contamination plays a more significant role. Several potential human

pathogens are naturally present in the intestinal tract of cattle and these animals do not show signs of infection.Environmental sources of contamination like personnel contamination may contribute significantly as a source of microbial contamination of milk during machine milking, although workers suffering from certain zoonoses, such as Q fever, may pose a potential risk (11).Air is also thought to be an insignificant contributor to microbial contamination of raw milk. It has been calculated that airborne bacteria account for <5 cfu/ml of the bacterial load of milk; of these Bacillus spores would constitute <1 cfu/ml (12). However, a recent study by Pangloli et al. (13) suggests that milking parlor air is a major source of Salmonella on the dairy farm.Water used in the production of milk may be one of the problems and arise when untreated water supplies are used to rinse and wash equipment. Such water may contain a diverse array of microorganisms including Pseudomonas spp., coliforms, Bacillus spp. and numerous other types of bacteria (14).Contamination from milking and storage equipment could play a significant contamination of milk can arise from inadequately sanitized surfaces of milking and milk storage equipment. McKinnon et al. (15) demonstrated that the total bacterial count of milk may increase by up to 3 _ 103 cfu/ml due to milking equipment and by a further 1:5 _ 103 cfu/ml from the bulk tank.In the food industry, preparations containing germicides are used on the udder to reduce the carriage of potential pathogens(16). Moist heat (water at below $100C^{o}$) could be used for disinfection processes (17). Iodophore, hibitane and potassium permanganate are all used in udder washing. Iodine solutions or tinctures long have been used by health professionals primarily as antiseptics on skin, and used both as antiseptics and disinfectants. An iodophor is a combination of iodine and a solubilizing agent or carrier. This product and other iodophors retain the germicidal efficacy of iodine but unlike iodine generally are non staining and relatively free of toxicity and irritancy (18,19). Chlorhexidine (hibitane) is an antiseptic effective against a wide variety of gram-negative and gram-positive organisms, facultative anaerobes, aerobes, and yeast. Chlorhexidine is used as an ingredient of bacteriostatic and bacteriocidal in a general purpose of skin cleansers, germicidal hand rinses and animal disinfection products. (20). Chlorhexidine is an antiseptic with antibacterial, antifungal and some antiviral activity and used in teat dips. (21).

Materials and Methods

Udder treatments:

Udders were treated as follows:

1-Untreated udder ; 2- Wet towel washing ($55\text{-}60C^{0}$),followed by paper towel drying; 3- Wet towel washing ($55\text{-}60C^{0}$), then by 0.1:100 Iodophor, followed by paper towel drying; 4- Wet towel washing ($55\text{-}60C^{0}$), then by 0.5:100 Iodophor, followed by paper towel drying; 5- Wet towel washing ($55\text{-}60C^{0}$), then by 1:100 Iodophor, followed by paper towel drying; 6- Wet towel washing ($55\text{-}60C^{0}$), then by Hibitane at a concentration of 1% followed by paper towel drying; 7- Wet towel washing ($55\text{-}60C^{0}$), then by Hibitane at a concentration of 0. 5%, followed by paper towel drying; 8- Wet towel washing ($55\text{-}60C^{0}$), then by Hepitane at a concentration of 0.25%, followed by paper towel drying; 9- Wet towel washing ($55\text{-}60C^{0}$), then by half strength Lukewarm potassium permanganate 0.5:10000, followed by paper towel drying, followed by paper towel drying; 10- Wet towel washing ($55\text{-}60C^{0}$), then by Lukewarm potassium permanganate at a concentration 1:10000. All these treatments were applied onto udders for 30-60 seconds.

Sampling:

Eighty milk samples and 80 swab of treated and non-treated udders were collected from cows reared at the college of Agriculture and forestry, during the period

December 2007 to January 2008, according to (22) as follows:Sterile cotton swabs immersed in peptone saline solution were used for sampling. Swabs were streaked on five locations of an area of 16 cm^2 and placed in test tubes containing peptone saline, and transferred to the veterinary public health laboratory (College of veterinary medicine) in a cool box within hour for enumeration of CFU/swab or ml of milk on nutrient agar.Decimal serial dilution 10^{-1}-10^{-6} were prepared, and the method of John,1997 was followed for counting CFU/swab or milk according to the formula:

$$\text{CFU/swab or 1 ml of milk} = \frac{\text{No. Of calculated colonies} \times \frac{1}{\text{Dilution}}}{\text{Volume of diluents' used for spreading}}$$

Results

Colony forming units (CFUs) of udder swab samples:

The Log10 of CFU/ swab of untreated udders was significantly ($p \leq 0.05$) differ from those swab samples when udder was treated with wet towel washing (55-60C^0), followed by paper towel drying (Table 1),

Table 1: Log10 of CFU/swab of udder before and after water treatments

Treatment	Log10 CFU/swab before udder treatment	Log10 CFU/swab after udder treatment
T1	5.518 a	A 5.518 a
T2	5.633 a	B 3.602 b

By using iodophor, the results show that there was in general significant ($p \leq 0.05$) reduction in the Log10 of CFU/ swab before and after udder treatment with all used concentrations of iodophor, and there was also significant ($p \leq 0.05$) reduction in the Log10 of CFU/ swab between different iodophor concentrations, with a proportional reduction in the Log10 CFU/ swab with each increase in the Iodophor concentration(Table 2).

Table 2: Log10 of CFU/swab of udder before and after iodophor treatments

Treatment	Log10 CFU/swab before udder treatment	Log10 CFU/swab after udder treatment
T3	5.531 a	A 2.301 b
T4	5.556 a	B 1.150 b
T5	5.447 a	C 0.231 b

Treatments with hibitane and in all of its concentrations, it was shown that there was a significant ($p \leq 0.05$) reduction in the Log10 of CFU/ swab after udder treatments, and there were also proportional and significant ($p \leq 0.05$) reduction in Log10 CFU/ swab with each increase in hibitane concentration (Table 3).

Table 3: Log10 of CFU/swab of udder before and after hibitane treatments

Treatment	Log10 CFU/swab before udder treatment	Log10 CFU/swab after udder treatment
T6	5.505 a	C 0.298 b
T7	5.440 a	B 2.034 b
T8	5.491 a	A 3.176 b

Potassium permanganate have also as other above treatments a significant ($p \leq 0.05$) reduction effect in the Log10 of CFU/ swab after udder treatments, and also proportional and significant ($p \leq 0.05$) reduction in Log10 CFU/ swab of the udder with each increase in potassium permanganate concentrations (Table 4)

Table 4: Log10 of CFU/swab of udder before and after potassium permanganate treatments

Treatment	Log10 CFU/swab before udder treatment	Log10 CFU/swab after udder treatment
T9	5.716 a	A 3.146 b
T10	5.568 a	B 2.954 b

Colony forming units (CFUs) of milk samples:

The Log10 of CFU/ ml of milk of untreated udders was significantly ($p \leq 0.05$) differ from those milk samples when udder was treated with wet towel washing (55-60C^0), followed by paper towel drying , and in the same time there was significant difference between the Log10 of CFU/ ml of milk before and after udder treatment with wet towel washing (55-60C^0), followed by paper towel drying(Table 5),

Table 5: Log10 of CFU/ml of milk before and after water treatments

Treatment	Log10 CFU/ml of milk before udder treatment	Log10 CFU/ ml of milk after udder treatment
T1	3.342 a	A 3.342 a
T2	3.819 a	B 2.672 b

By using iodophor, the results show that there was in general significant ($p \leq 0.05$) reduction in the Log10 of CFU/ ml of milk before and after udder treatment with all used concentrations of iodofore, and there was also significant ($p \leq 0.05$) reduction in the Log10 of CFU/ ml of milk between different iodophor concentrations, with a proportional reduction in the Log10 CFU/ ml of milk with each increase in the iodophor concentration(Table 6).

Table 6: Log10 of CFU/ml of milk before and after iodophor treatments

Treatment	Log10 CFU/ml of milk before udder treatment	Log10 CFU/ ml of milk after udder treatment
T3	3.662 a	AB 1.602 b
T4	3.826 a	B 0.801 b
T5	3.556 a	A 0.160 b

Treatments with hibitane and in all of its concentrations, it was shown that there was a significant ($p \leq 0.05$) reduction in the Log10 of CFU/ ml of milk before and after udder treatments, and there were also significant ($p \leq 0.05$) reduction in Log10

CFU/ ml of milk with the highest hibitane concentration (3%), compared with the other lower two concentrations (0.75% and o.37%) (Table 3).

Table 7: Log10 of CFU/ml of milk before and after hibitane treatments

Treatment	Log10 CFU/ml of milk before udder treatment	Log10 CFU/ ml of milk after udder treatment
T6	3.785 a	B 2.00 b
T7	3.653 a	B 2.079 b
T8	3.838 a	A 3.380 b

Potassium permanganate treatments with its two concentrations showed no differences in Log10 /ml of milk before and after udder treatments or between its two concentrations (Table 8).

Treatment	Log10 CFU/ml of milk before udder treatment	Log10 CFU/ ml of milk after udder treatment
T9	3.994 a	A 3.591 ab
T10	3.997 a	A 3.554 ab

percentage reduction of Log10 CFU/ swab of udder surface in different treatments

The lowest percentage in the reduction of Log10 /swab was noticed when wet towel washing (55-60C^0), followed by paper towel drying being 36.05%. By using iodophor there was an increase of about 20% in the reduction of CFU/swab with each increase in iodophor concentration, and were 58.5%,79.3% and 95.7% with concentrations of 1:100, 0.5:100 and 0.1:100). The highest concentration of hibitane gave a reduction in CFU/swab similar to that of the higest iodophor concentration, being 94.5%.A decline in the percentage of CFU/swab was proportional with the decrease in hibitane concentration, being 62.6% and 42.1% , when hibitane was used at concentrations of 0.5% and 0.25% respectively. Potassium permanganate treatments show a slightly better than wet towel treatments, being 44.9% and 46.9% in concentrations 0.5:10000 and 1:10000 respectively.

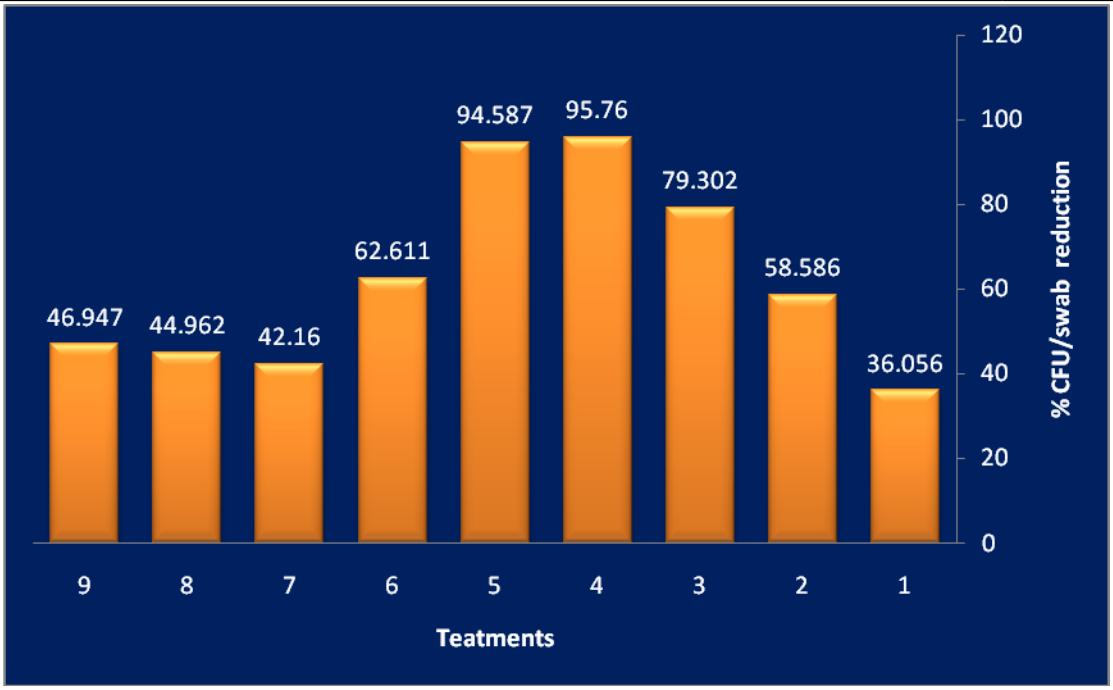

Figure 1: Percentage reduction of Log10 CFU/ swab of udder surface in different treatments

Reduction percentage of LOG10 CFU/ ml of milk in different treatments:

The lowest percentage in the reduction of Log10 /swab was noticed when wet towel washing (55-60C^0), followed by paper towel drying being 30.0%. By using iodophor there was an increase of about 20% in the reduction of CFU/swab with each increase in iodophor concentration, and were 56.2.5%,79.0% and 95.5% with concentrations of 1:100, 0.5:100 and 0.1:100). Hibitane concentrations gave a reduction in CFU/swab lower than that given by iodophor, being 75.4%.A decline in the percentage of CFU/swab was proportional with the decrease in hibitane concentration, being 47..3% and 39.5% , when hibitane was used at concentrations of 0.5% and 0.25% respectively. Potassium permanganate treatments show a slightly better than wet towel treatments, being 40.5% and 49.7% in concentrations of 05.10000 and 1:10000 respectively.

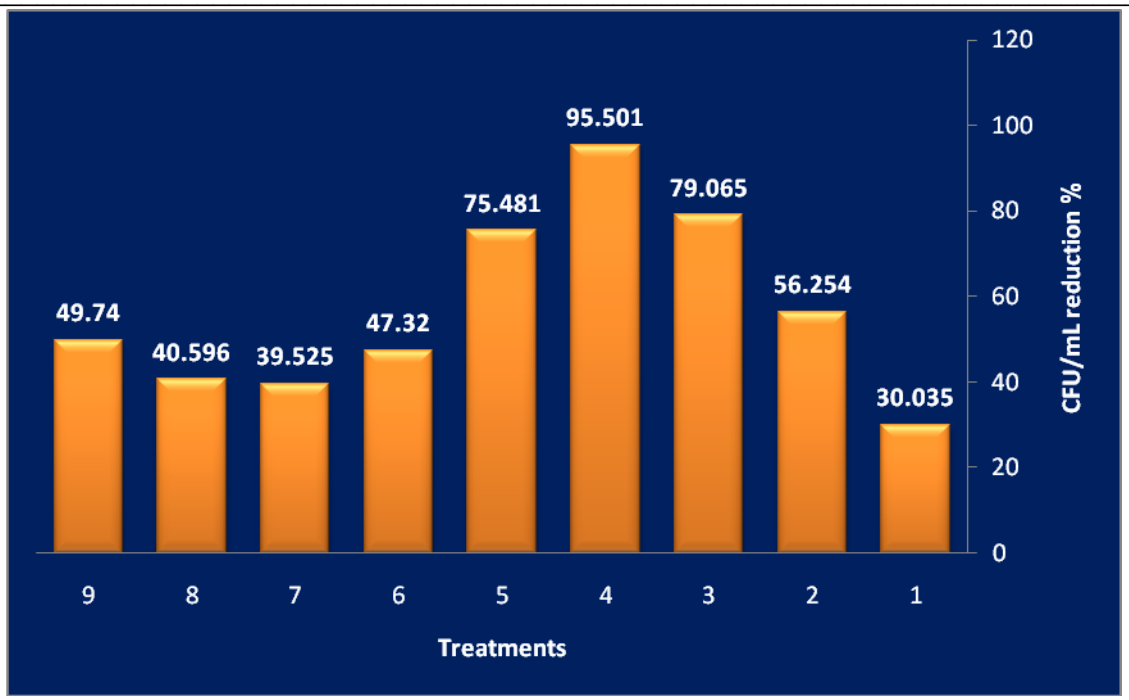

Figure 2: Percentage reduction of Log10 CFU/ ml of milk in different treatments

Discussion

According to the field of application, strategies for the prevention of the transfer of microbial skin flora from the udders must consider the various categories of flora: transient, resident or stemming from infected lesions on the udder (infection flora). Depending on the species and virulence of the microorganism and of the susceptibility of the infection target, transient flora may or may not be of pathogenic importance. In contrast, resident skin flora is usually regarded as pathogenic. Microorganisms stemming from infected lesions are of proven pathogenicity. only the transient and infection flora from the udders play a role. Milk may be rendered safe by procedures for the elimination of transients such as hygienic udder wash by hand rub (in the order of increasing efficacy). Furthermore, the duration of treatment (between 30 and 60 s) significantly influences the achievable reduction of microbial release. According to the new European standards (CEN) for testing chemical disinfectants and antiseptics, products for hygienic udder wash must be significantly more efficacious than contaminated udders. By this, the average reduction of microbial release amounts to 4.2 to 4.4 lg, in udder surfaces.. There exists a strong positive correlation of the reduction of microbial release in milk and the duration of udder treatment. Some of them exert a bacteriostatic sustaining This, however, is not necessary with the latter as the initial bacterial reduction is that strong that restitution of the udder skin flora takes > 3 hours (23).The results of this study showed that surface treatment of udder with towel moistened in warm water at 55-60Co was able to reduce CFU/swab by two Log2 only, which is the lowest reduction (36%) of udder contaminating flora among all other treatments. This temperature is used for disinfecting serum and vaccines from contaminating bacteria. Although there was a significant reduction in CFU/swab in treatment with water at this temperature, compared to pre-treatment CFU/swab, but it should be said that not all contaminating bacteria were completely eliminated through the action of coagulation and denaturation of bacterial proteins, since it should be worked

for 1 hour to inactivate vegetative bacteria but not spores (17)**.** In this study we prepare more concentrated dilusions to compensate the shorter period of contact on udder surfaces, in order not to take more time waiting for milking. The best results in reduction of udder contaminating flora was achieved by applying iodophor , compared to other chemicals used. There was 5,4 and 3 Log10 reduction in CFU/swab of udder contaminating bacteria when iodophor was used at 1:100, 0.5:100 and 0.1:100 concentrations when comparison is made between pre and post treated udders. The percentage of reduction was proportional with each increase in iodophor concentration, from 58.5% to 95.7%. Published reports on the in vitro antimicrobial efficacy of iodophors demonstrate that iodophors are bactericidal, mycobactericidal, and virucidal (24). Three brands of povidone-iodine solution have demonstrated more rapid kill (seconds to minutes) of *S. aureus* and *M. chelonae* at a 1:100 dilution than did the stock solution (25). Iodine compounds have the widest spectrum of antiinfectives against bacteria, fungi, spores, protozoa, viruses, and yeasts. Aqueous iodine are less effective than alcoholic solutions, but alcoholic component is drying and irritating to abraided skin. Iodophor is convenient to use as it is less irritating, but not as effective. In the second order was with hibitane application, and as Chlorhexidine is used as a safe antiseptic or disinfectant to apply to prevent body infection in the form of acetate, gluconate or hydrochloride, so it is in this study it gave a reduction of 5, 3 and 2 Log10 of CFU/swab of the contaminating udder flora, was noticed by using 1, 0.5 and 0.25% of hibitane treatment of udder surface. The percentage of CFU /swab reduction were 94.5%, 62.6 % And 42.1% respectively. In clinics, skin germicides are used to reduces kin carriage of potential pathogenic bacteria like chlorhexidine (Hibitane) and iodofors are used, and chlorhexidine in food premises appear to reduce skin bacteria and carriage of potential pathogens (16). Antimicrobial activity of 0.2%, 1%, and 2% chlorhexidine gluconate was tested against Staphylococcus aureus and Candida albicans, and found that 2.0% gel and liquid formulations eliminated Staphylococcus aureus and Candida albicans in 15 seconds, whereas the gel formulation killed Enterococcus faecalis in 1 minute(26). , By this, the achievable average reduction of the microbial release ranges between 2.0 and 2.4 lg. In contrast, antiseptic washing procedures with preparations containing low concentrations of iodophore, chlorhexidine gluconate reduce the bacterial release within 2-5 min only by 0.5 to 1.2 lg(23). The efficacy of iodophor germicides containing different concentrations of available iodine against natural udder microflora when compared with chlorhexidine gluconate (0.25 to 1%) liquid detergent (Hibitane), non-germicidal hot water rinse. The tap water rinse was ineffective compared with all other treatments, because it reduce CFU/swab of contaminating bacteria only 2Log10, and donot reach 4Log10 reduction as iodophore and hibitane . Only 1% chlorhexidine gluconate liquid detergent and iodophor at 1:100 concentration were significantly better than other concentration of both chemicals, These agents caused a significant reduction in the number of 'natural' microorganisms released from udder after a standard 30-60 s udder wash. The low-concentration iodophor products and chlorhexidine gluconate failed to give satisfactory results of 4.2 to 4.4 Log reduction in CFU/swab of natural udder contaminating bacteria, since they should be applied for more longer period of contact,2-3 minutes (27) . The germicidal effects of potassium permanganate and eosin were not satisfactory, since they were near to that of warm water. This could be due to short contact time of 30-60s instead of 2-3 minutes. The picture of CFU/ml of milk is a mirror to that of CFU /swab on the udder

surface. A slightly similar picture was also obtained in the percentage of reduction in CFU/ml of milk. Wet towels treatment was effective in reducing CFU/ml of milk, with a percentage of 30.0 % . The higher concentration of iodophor (1:1000), was the one which significantly reduce CFU/ml of milk (95.5%), among the other two concentration which were similar in their significance reduction of CFU/ml of milk (79.0 and 56.2 respectively). The same picture in CFU/swab reduction was noticed through using different concentrations of hibitane to that of iodophore, but the difference was in the rate of reduction which was higher in case of iodophore than that recorded for hibitane. No significant differences in the reduction of CFU/ml of milk or in the percentage of this reduction was reviled by using potassium permanganate in the hygienic treatment of udder before milking. In conclusion, it is clear that iodophor is the most effective among the three chemicals used, although all of them can be used for udder washing before milking process. The application of these chemicals in hygienic treatment of udder surfaces are worth to be applied in the practical milking process.

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تاثيرتهيئة الضرع على نظافته ونوعية الحليب المنتج

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الخلاصة

تم إجراء العد البكتيري الكلي ل 80 عينة الحليب و 80 مسحة لضرع الابقار تم جمعها من 20 بقرة وذلك لمقارنة تأثير معاملة الضرع بغسله بمنشفة مبللة (55 -60^{0}C)، تلاها التجفيف بمنشفة ورقية، وثلاثة تركيزات للمعقمات Iodophre وHibitane تلاها التجفيف بمنشفة ورقية وتركيزين من برمنكنات البوتاسيوم تلاها التجفيف بمنشفة ورقية مع ضرع غير معالج. اظهرت النتائج أن LOG10 للوحدات المكونة للمستعمرة / مسحة للضرع غير المعالج تختلف معنويا ($P \geq 0.05$) عن تلك المسحات المعالجة للضرع بالغسل بمنشفة مبللة (55 - 60^{0}C)، تليها التجفيف منشفة ورقية. وباستخدام اليود، كان هناك في اختلاف ملحوظ ($P \geq 0.05$) في LOG10 للوحدات المكونة للمستعمرة / مسحة بعد العلاج الضرع مع جميع التركيزات المستخدمة من اليود، وكان هناك أيضا انخفاض نسبي في LOG10 للوحدات المكونة للمستعمرة / مسحة مع كل زيادة في تركيز اليود (0.1:100، و0.5:100 1:100) على التوالي. وقد اكتسب Hibitane بتركيزات 0.25٪، 0.5٪ و 1٪ نفس الصورة عند استخدام Iodophre. وأظهرت المعاملة ببرمنغنات البوتاسيوم بتركيزين اثنين وجود فروق في LOG10 للوحدات المكونة للمستعمرة / مل من الحليب قبل وبعد معاملة الضرع بالتركيزات 0.5:1000 و1:1000. العد البكتيري الكلي للعينات الحليب كانت مرآة لعينات مسحات الضرع. من النتائج اتضح أن المطهرات المستخدمة أو الماء الساخن كانت جميعها فعالة في الحد من تلوث الضرع قبل الحلب.