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The Influence of Udder Sanitation on Hygienic Quality of Cow Milk in and Around Addis Ababa

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Abstract: The aim of the research was to determine the significance of udder disinfection after milking on the hygienic quality of fresh raw milk in dairy cowherds. The research was conducted on selected four farms that are found in and around Addis Ababa with differing hygienic milk quality, during which one farm was selected for the assessment of udder disinfection after milking and the other three farms to implement primary hygiene with water in the preparation of udder before milking. The disinfection in the first group after milking was performed by immersing the teats in a special cup containing AUDIP, Monopropylenglycol 2% solution. Seven round individual sampling of milk were collected from each cow within three months for determining total microorganism and Somatic cell counts and the result showed ($P<0.05$, $x^2= 36.750$) for the total viable count of the first group. There was insignificance P-value for the total somatic cell count of the first group ($P=0.369$, $x^2 =13$) even though there was somewhat promoted decrease in the total somatic cell numbers during the course of trial. In addition, results obtained from out of the three group, one showed (Woldesilassie farm)that a slight significant reduction in total viable count ($P= 0.015$, $x^2 = 33.298$) but for the total somatic cell count insignificant P-value was recorded ($P= 0.850$, $x^2 = 16.962$). The other two groups out of the three showed no significant change in both total viable and somatic cell count (Biofarm and Tsegenet farm).

Key words: Disinfection · Milk · Sanitation · Somatic cell count · Teat · Total viable count and Udder

INTRODUCTION

Livestock constitutes one of the principal means of achieving living standards in many developing world. In sub-Saharan African countries, livestock plays a crucial role in both natural economies and the livelihood of rural communities. It provides drought power, milk, meat, and input for crop production, soil fertility and raw material for industries[1]. As in many countries livestock, particularly cattle play multiple roles in Ethiopia being a source of milk, meat, hides and other products and byproducts [2].

The estimated small and large ruminant population in Ethiopia is 40.3 million cattle, 20.7 million sheep and 16.2 million goats[3]. Milk is the lacteal secretion of the mammary glands of a mammal. As it is well known, milk is the first natural food of all young mammals during the period immediately after birth [4]. Man has consumed milk and milk products even before the dawn of civilization. Because of its high nutritive value, milk is considered as one of the most important diet items of many people[5]. Nutritionally, milk has been defined as "the most nearly perfect food". It provides more essentialnutrients insignificant amounts than any other single food. Milk is an outstanding source of calcium and phosphorus for bones and teeth, and contains riboflavin, vitamin B_6, A and B_1 in significant amounts. It also contains vitamin B_{12}, the antipernicious anemia vitamin [6].

As milk and milk products play an important role in human nutrition throughout the world. Consequently, the products must be of high hygienic quality. In less developed areas and especially in hot tropics high quality of safe product is most important but not easily accomplished [7]. This is required since milk is also a suitable substrate for microbial growth and development. The fluid or semi fluid nature of milk and its chemical composition (containing the essential nutrients) renders it as one of the ideal culture media for microbial growth and multiplication [4, 8-10].

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Mainly because of this reason, milk and milk products are more prone to the harboring and proliferation of microorganisms. Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted in to the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources i.e., within the udder, the exterior of the udder and the surface of the milk handling and storage equipment [11, 12]. Therefore, to keep the udder of milk herds clean and healthy, it is important to carry out hygiene-prophylactic measures prior to and after milking [13].

Nowadays, there are different disinfectants for udder hygiene available but the ecologically acceptable agents with a high degree of biodegradability and not skin aggressive ones are preferred [14]. Building in which animals are housed should be adequately spacious, warm, lit and ventilated for the maintenance of animalhealth. Bedded and lying areas and passageways should be kept clean so that animals and especially udders do not become heavily soiled between milking. Stalls, standings or bedded areas should be long enough to minimize soiling of the udder [13].

The International Dairy Federation (IDF) has worked out a general acceptance. The code gives high importance to the production of milk with a good bacteriological quality [13]. So far, there are no available studies about the relation between udder sanitation on the quality of cow milk in Ethiopia . With respect to public health hazard and those of zoonotic diseases, it is very crucial to investigate the influence of udder sanitation on hygienic quality of cow milk. Since there are several diseases which are transmitted to human through the consumption of unhygienic or contaminated raw milk, this study was performed with the aim of filling the gap that has been created and aids in the contribution for the community health. Therefore, the present study was conducted to provide adequate and scientific information on the relationship between udder sanitation and milk quality and to determine the effect of udder and teat cleaning (disinfection) on somatic cell count and total microorganisms count in the dairy herds.

MATERIALS AND METHODS

Study Area: The study was conducted from December, 2008 to April, 2009 in Addis Ababa. The area receives mean annual rainfall of 1300mm in bimodal distribution, with minimum and maximum temperature of 24 °C and 11 °C respectively. The long rainy season extends from June to September followed by a dry season ranging from October to February. The short rainy season lasts from March to May [15].

Study Population: In this research, 31 cross and Holstein Friesian dairy cows were involved from the selected four dairy farms that are located in and around Addis Ababa in the year 2008/2009.

Study Design: The investigation was conducted on selected four dairy farms in and around Addis Ababa to determine somatic cell count and total microorganisms count. In the farms subjected to analysis, a survey was conducted during visits, the aim of which was to determine the method of preparing the udder and teats for milking (sanitation protocol). The first farm was subjected to disinfected agents after milking by immersing teats in a special cup, containing (AUDIP,monopropylene glycol 2% solution) and washing the teats and udders prior to milking with Luke warm water and wiping with individual use dry towel. The other three small farms practices udder and teat cleaning prior to milking based on washing with warm water and wiping with disposable clothes and they did not utilize any means of disinfections before or after milking. The usual milking procedure was conducted twice a day in all farms with the use of manual milking.

Study Protocol: Milking samples were collected from all groups. First sampling was done on day 0 to determine the nominal condition and after the introduction of disinfecting agents every 14 days throughout a period almost 3 months. Individual milk sample from each cow was used in the investigation to determine somatic cell and microorganism counts. Each sample was an equal quantity of milk obtained from every quarter of the udders and collected in a 15-20ml sterile test tube immediately after squeezing the first gushes of milk into a separate dish. The sample was delivered to the laboratory in an icebox at around 4°C and laboratory examination done within 1-hour interval.

Sampling Method: Experimental study protocol was conducted during the determination of the somatic and total viable count in those selected farms. The somatic cell counts were determined in directly using CMT equivalent (17). After the reaction of the sample and the reagent observed the test result was known according to the standard equivalent somatic cell count. In addition, basic dilution of samples was created by using Indian standards for total viablecount, cultured using plate count agar

Table 1: California Mastitis Test reactions and Equivalent milk Somatic Cell Counts

Test results	Reaction observed	Equivalent milk SCC
Negative	The texture remains fluid without thickening or getFormation.	0-200000 cells/ml
Trace	A slight slime formation is observed. this reaction is most noticeable when the paddle is rocked from side to side.	150000-500000 cells/ml
1+	Distinct slime formation occurs immediately after mixing solutions. This slime may dissipate over time. when the paddle is swirled, fluid neither forms a peripheral mass nor does the surface of solution Become convex or 'domed up'.	400000-1500000 cells/ml
2+	Distinct slime formation occurs immediately after mixing solutions. When the paddle is swirled, the fluid forms a peripheral mass and the bottom of the cup is Exposed.	500000-5000000 cells/ml
3+	Distinct slime formation occurs immediately after mixing solutions. This slime may dissipate over time. When the paddle is swirled the surface of the Solution becomes convex or 'domed up'.	>5000000 cells/ml

Source: [17]

Table 2: Bureau of Indian Standards (BIS) for viable plate counts

Total Microbial Count	Grading
$<2x10^5$ cfu/ml	Very good
$2x10^5$cfu/ml……..$10x10^5$cfu/ml	Good
$10x10^5$cfu/ml……$50x10^5$cfu/ml	Fair
$>50x10^5$cfu/ml	Poor

Source:[18]

medium in petri dishes and incubated at 37 °C for 24 hours from which the total number of colonies were recorded with the counter. The total microorganism count (TMC) was determined with a standard plate count method (17). The number of colonies obtained represented the number of live microorganisms in 1ml of milk.

Somatic Cell Count Determination: The somatic cell members will be determined indirectly by the California mastitis test(CMT). The CMT is based on detecting the presence of DNA in the milk sample. Thus, it detects somatic cells both leukocytes and epithelial cells. The CMT accurately reflects the leukocytes and epithelial cells count in milk, with the exception of early lactation and toward the end of lactation, when high epithelial cell counts may give false positive reactions [16].

Total Viable Count (TVC): The standard plate count (SPC) method was used in assessing the number of viable bacteria in milk based on which cow milk can be graded in to different categories according to bacterial content in it. Appropriate dilutions of milk sample were plated in to plate count agar medium in Petri dishes. Up on incubation, the bacteria present in the sample multiplied and formed visible colonies, which can be counted. The number of colony forming units (CFU), multiplied by dilution factor gives SPC per ml of milk. Recording of the data or results was done after selecting two Petri dishes of the same dilution having colonies between 30 and 300 in number. The number of colonies obtained will represent the number of live microorganism in 1ml of milk. Grading of milk sample obtained from the four farms was done depending up on the standard plate count method, According to the bureau of Indian standards (BIS) (18).

Data Analysis: Data were entered in to Microsoft excel spreadsheet and basic statistical analysis of the collected data was performed using SPSS version 10.0 statistical. Chi-square analysis was used to determine association of hygienic condition with somatic cell and total viable counts. P-values less than 0.05 ($p<0.05$) were considered as the presence of statistical significant association.

RESULTS

As it is evident from Table 3, the somatic cell count in the first group (genesis farm) was85.8% (0- 200,000 cell/ml), 8.2 % (1.5* 10^5-5*10^5) and 6% (4*10^5-1.5*10^6) and at day 0 only 9 samples were between 0-200,000 cell/ml, was decreasing through the course of trial reaching up to 14 and a total of 84 samples were recorded in the range between 0-200,000 cell/ml. Even though there is promoted decline in the somatic cell count/SCC, this value is not statistically significant ($p=0.369$) (Table 4).

In the other three farms, there was a slight variation in the SCC, and high number of SCC was recovered in these groups during the course of trial than in the first group. More detailed monitoring of the SCC including the mean, SD, P-value, and chi-square in individual group is presented in Table 3 and 4.

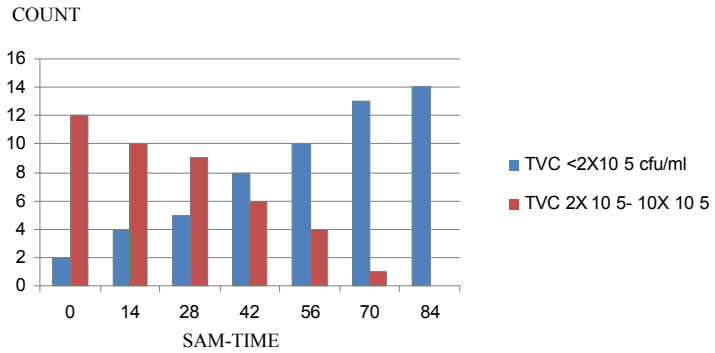

Fig. 1: Monitoring of Total Viable Counts in the study Group 1 (Genesis); with 95% confidence interval.

Table 3: Percentages of the Total Somatic Cell Count for Individual study group

SCC	Group 1, n= 14	Group2,n=6	Group3,n=7	Group4,n=4
0-200,000cell/ml	85.8%	16.7%	26.5%	7.1%
150,000-500,000cell/ml	8.2%	7.1%	12.2%	3.6%
400,000-1,500,000cell/ml	6.0%	26.2%	24.5%	57.1%
500,000-5,000,000cell/ml	-	23.8%	24.5%	10.7%
>5,000,000cell/ml	-	26.2%	12.2%	21.4%
Total	100%	100%	100%	100%

Table 4: The Mean, SD, P-value and Chi-square of SCC for individual study group

SCC	Group 1(Genesis) n= 14	Group2(Biofarm) n=6	Group3 (Weld.) n=7	Group4 (Tseg.) n=4
Mean	0.20	2.36	1.84	2.36
SD	0.54	1.39	1.39	1.10
P-value	0.369	1.00	0.850	0.827
Chi-square	13	7.382	16.962	17.500

Table 5: Percentage of the Total Viable Count /TVC for individual study group

TVC	Group 1(Genesis) n= 14	Group2(Biofarm) n=6	Group3 (Weld.) n=7	Group4 (Tseg.) n=4
$<2x10^5$cfu/ml	57.1 %	9.5%	12.2%	7.1%
$2x10^5$-$10x10^5$cfu/ml	42.9%	45.2%	34.7%	7.1%
$10x10^5$-$50x10^5$cfu/ml	-	45.2%	51.0%	64.3%
$>50x10^5$ cfu/ml	-	-	2.0%	21.4%
Total	100%	100%	100%	100%

Table 6: The Mean, SD, P-value and Chi-square of TVC for individual study group

TVC	Group 1(Genesis) n= 14	Group2(Biofarm) n=6	Group3 (Weld.) n=7	Group4 (Tseg.) n=4
Mean	0.43	1.36	1.43	2.00
SD	0.50	0.66	0.74	0.77
P-value	0.00	0.337	0.015	0.320
Chi-square	36.750	13.447	33.298	20.222

The total viable count/TVC in the first group was 57.1% ($<2x10^5$cfu/ml) and 42.9% between $2x10^5$cfu/ml and $10x10^5$cfu/ml and there was gradual decrement in the total viable count of this group during the course of trial starting from day zero (0) up to day eighty four (84) (Figure 1).

The total viable counts in the other three groups were quite different from the SCC result. There was an increment in TVC throughout the course of trial from day zero (0) up to 84 in the two groups (Biofarm and Tsegenet farm). However, one group (Woldesilassie farm) out of the three has a P-value of 0.015 (Table 6) and there was gradual decline of TVC throughout the course of trial from day zero (0) up to 84. More detailed monitoring of the TVC in the first group is presented including individual group mean, SD, P-value, and Chi-square in Table 5 and 6.

DISCUSSION

As far back as the end of last century most of the members of international milk society considered the udder preparation for milking was to be washed with water and to dry them with washcloth [19]. Still it has been proved that, such a way does not reduce enough post

secretory milk contamination; moreover, it does not adequately affect the milk gland state of health [20]. Thus, the disinfection should be done prior to milking, since it reduces the total number of bacteria from skin of teats significantly [21]and improves the hygienic quality of milk [22].

In the same time, the frequency of intra mammary infections caused by environment infective agents is reduced by disinfecting the teats after milking [23].

The effect of certain disinfection agents on hygienic milk quality and udder health status has been evaluated in many studies. Teat dipping with a germicidal solution immediately after every milking has proved to be an effective milking management practice to reduce the rate of new intra mammary infections, substantial evidence supports post milking teat antisepsis as the single most effective practice in the preventive of new contagion IMI in lactating dairy cows [24].

Teat dipping is a simple, effective, and economical means to reduce bacterial population on teat skin. An effective teat dip, (AUDIP, Monopropylenglycol 2% solution) correctly used, can reduce the incidence of new udder infection by 50 to 90% [25].However, it seems that the reduction of infection risk causes a drop in somatic cell and microorganism count, which was also observed with the use of other disinfecting agents [26].According to the data obtained, it was evident that the somatic cell count in the first group demonstrates a tendency to decrease in comparison to the starting values, but this value is not statistically significant ($P>0.05$).

In contrast although somatic cell counts in the other three groups demonstrated a continuous increase in such a degree from the beginning until the end of the trial. However, it was not significantly larger in relation to the somatic cell number at the beginning of the trial. Because it showed a slight fluctuation in P value of ($p=1.00$, 0.850, and 0.827 for group two, three and four respectively). By observing the total viable microorganism count (TVC) in the cow's milk of the first group (Genesis), it was evident that, there is a decreasing trend during which there is a statistically significant reduction from the beginning of the trial continuing to the end of the investigation.

In contrast, milk from the other three groups whose udders were treated with water, showed no significant difference in the TVC because of the slight oscillations in these values except that the third group (Weldselassie's farm). Where there was significant reduction showed up to the end of the trial. Because it has been demonstrated that washing the udder with water decrease the microorganism number on teat skin by 54.5-57.1% [21, 22].

By an overall assessment of the trial results obtained from the first group, it is clear that, the statistical insignificant in the total somatic count, was due to the correct interpretation of SCC data depends on understanding of the factors that influence the number of cell in milk at the quarters, cow or herd level [24].

The insignificant values of the SCC in the first group could be due to the factors other than infection, can cause a substantial SCC increase, chemical irritants, such as antibiotic therapy, can cause raised SCC. Trauma to the udder can also cause higher than normal SCC levels. Experimentally induced stress on individual cows has results in higher SCC. The time and method of sampling may affect the resulting SCC value. For example, counts are lowest immediately before routine milking and are highest in stripping sample.

Samples at evening milking are somewhat higher than in the morning. There is considerable variation in SCC of an individual cow from day to day. The reason for this diurnal variation includes fluctuations in production, spontaneous eliminationof new infections, unobserved udder injury, stress, and inherent biological variation of individual cows [24].

In addition, the CMT accurately reflects the leukocytes and epithelial cells count in milk, with the exception of early lactation and toward the end of lactation, when high epithelial cell counts may give false positive reactions [16].

[27], [28] and [29] indicated that although fore stripping ispotentially veryeffective method of lowering the SCC, at the same time the procedure may increase the frequency of IMI due to the close contact of the milker's hand with the teat.[30]Claimed that the milker's hand has to be both dirty and wet for the infections to spread in this way.

Generally, in this study a significant reduction in TVC of the first group (Genesis) have been obtained and the third group that had been exposed to the udder and teat cleaning using water and wiping with a dry towel have shown significantly reduction in the TVC. Corresponding relationships between the method of udder and teat preparation for milking and TMC were also observed by [31], [32] and [33] who showed a higher microbiological quality of milk in the herds where cleaning with a dry cloth was practiced in comparison with the herds where other methods were adopted.

It is believed that, a low TMC level in the milk of cows, in which cleaning the udder and teats with a dry cloth is employed, results from the fact that this method of pre-milking preparation inhibits the transfer of

microorganisms from the central and upper part of the teat, as well as from the udder to the lower section of the teat and as a consequence, to the milked milk [34].

Although, the SCC in the first group have been affected by the disinfecting agents, but the total viable count was significantly affected by the agent, and in the other three groups also showed quiet variation in comparison to the first group and therefore, it is clear that they confirmed current findings that without disinfection at least after milking the milk obtained can be of poor quality and unsuitable for processing [35].

Besides, the results obtained from the first group it is in agreement with current studies of sanitation in milk hygiene, where it has been established that implementation of disinfecting agents in udder hygiene after milking can significantly reduce the average microorganism count in fresh raw milk [21]. This effectively improves the microbiologic quality of the milk in a relatively short time, with the proviso that other sanitation procedures, including, sanitation of milking equipment, are conducted in primary milk production [36].

CONCLUSIONS AND RECOMMENDATIONS

As far as the research was conducted to meet its objectives all the necessary inputs and procedures were incorporated. The research result implicates that for milk bacterial quality udder sanitation based on udder and teats disinfection after every milking is indicated. The result obtained from the first group showed how disinfecting agents gradually reduces the number of total microorganisms in the cow milk that either contaminate the milk from udder surface or cause intra mammary infection inside the milk gland. Even if, the third group result showed somewhat reduction as compared to the first group in total viable count results, most studies that have been done so far recommend that washing the udder and teats with water certainly not in accordance with proper udder hygiene. So that, udder and teat disinfection has been implicated the most crucial inputs for safe and quality milk provision in many studies, and also this study reinforce those researches that have been done so far on this issue.

Based on the above conclusion the following recommendations are forwarded;

- Disinfecting agents promote the quality of raw milk by reducing the total viable count and should be implemented in small and large dairy farms.
- Udder disinfecting agents have a great role in mastitis control since they significantly reduce the bacterial load on udder skin and prevent the dairy cows from intra mammary infections.
- Many zoonotic diseases can be transmitted from animal to human via the consumption of raw milk. So that, tuberculosis and other milk borne infection risk can be reduced by frequent implementation of udder and teat disinfection with the available agents with high degree of efficacy on the quality of fresh raw milk.

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