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Original Scientific Article

BACTERIOLOGICAL QUALITY AND SAFETY OF RAW COW'S MILK AND FRESH CREAM

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Summary: In this study, the bacteriological quality and the presence of *S. aureus* and *E. coli* O157 in raw milk and fresh cream were studied. A total of 80 samples (38 raw milk, 38 fresh cream and 4 separator samples) were collected from June to August 2011 at a milk separation center. Samples were analyzed for Standard Plate Count (SPC), total coliforms, faecal coliforms, *E. coli* and *S. aureus* counts as well as for the pathogen *E. coli* O157. Means of counts per ml of milk for SPC, total coliforms, faecal coliforms, *E. coli* and *S. aureus* were 3.62×10^7, 1.65×10^6, 3.69×10^5, 2.83×10^4 and 4.68×10^3 cfu and per ml of cream were 7.79×10^7, 4.21×10^6, 2.07×10^6, 1.89×10^5 and 3.5×10^4 cfu, respectively. *E. coli* and *S. aureus* were isolated from 52.6 and 23.7% of raw milk samples, 47.4 and 31.6 % of fresh cream samples and 75 and 25 % of separators samples, respectively. *E.coli* O157 was detected in 2.6% of raw milk samples. The mean values of titratable acidity for raw milk, fresh cream and separators samples were 0.18, 0.20 and 0.24 %, respectively. Separators play a major role in the increase of the cream microbiota. High microbial counts of both raw milk and fresh cream may present a public health hazard to the consumers and emphasizes the need for improved hygienic standards.

Key words: milk; cream; *S. aureus*; *E. coli* O157; separators

Introduction

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. Fresh milk drawn from a healthy cow normally contains a low microbial load (less than 1000 cfu/ml milk) but the load may increase up to 100 times fold, or more, once it is stored for sometime at normal temperature (1). Bacteria in raw milk can occur through colonization of the teat canal or an infected udder (clinical and subclinical mastitis) or milk can get contaminated by the surface of the teats, air, milker (manual milking), water and milk contact surfaces, storage and transport equipment (2, 3).

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In Egypt, direct consumption of raw milk is much frequent and more popular than consumption of pasteurized milk because it is believed, especially in rural areas, that raw milk and its byproducts have nutritional advantages over the pasteurized one. However, consumption of raw milk and its byproducts is considered potentially hazardous and has been associated with several types of infections including brucellosis, tuberculosis, salmonellosis, yersiniosis, Escherichia coli O157 and Staphylococcal enterotoxin poisoning (4).

The main producers of milk in Beni-Suef governorate are small farmers with between one and four heads of cattle. There are only a few organized modern and large scale farms. Most farmers milk their cows manually and separate milk into cream and skimmed milk. Raw skimmed milk is used to manufacture Kareish cheese, while

fresh cream is stored at room temperature in a one piece goat skin bag to make butter. Both are sold in a market-place held once a week in each village.

Raw milk (or cream) is the basic material from which all dairy products are made. The diversity of microorganisms and the level of contamination in the raw material has a decisive effect on the quality and safety of the final product. Several studies have been carried out in Egypt to evaluate the bacteriological quality and safety of raw milk and fresh cream (5-10).

Since recent information concerning the bacteriological quality and safety of raw milk and fresh cream in Beni-Suef governorate is sketchy or totally absent, this study was carried out to investigate the bacteriological quality and safety of locally produced raw milk and fresh cream in Beni-Suef governorate.

Material and methods

I. Collections of Samples

The study was conducted in a village milk separation center (containing 4 hand operated separators) in Beni-Suef governorate, Egypt, where milk is manually separated into cream and skimmed milk. Everyday milk separation is performed at room temperature (20- 25 °C), between 6:00 and 10:00 a.m.

A total of 80 samples (38 raw milk, 38 fresh cream and 4 separators samples) was collected from June to August 2011 at the milk separation center. After agitation, milk and cream samples (150 ml) were taken aseptically from farmers containers and stored in sterile screw bottles. After agitation, separators samples (100ml) were obtained from separator bowl "remnants of milk, skim milk and cream", and collected in sterile screw bottles. Samples were transported to the laboratory in an insulated ice box (4 - 6 °C) within 1-2 h of collection and analyzed immediately upon arrival.

II. Bacteriological Analysis

(a) Preparation of Samples: Samples were diluted in 0.1% peptone water (Oxoid, UK) (11 mL of samples in 99 mL of 0.1% peptone water for initial dilution), subsequent decimal dilutions up to 10^7 were prepared with the same diluent and appropriate dilutions were used to enumerate the different groups of microorganisms.

(b) Standard Plate Counts (SPC): SPC was carried out using plate count agar (Oxoid, UK) for 48 ± 3 h after incubation at 32 ± 1 °C (11).

(c) Coliforms, Faecal Coliforms and Escherichia coli: Counting was estimated by a three tube Most Probable Number (MPN) technique (12).

(d) Enumeration, Isolation and Identification of *S. aureus*: *S. aureus* were enumerated by surface spread technique onto Baird Parker agar (Oxoid, UK) (13).

(e) Isolation and Identification of Escherichia coli O157: Twenty five milliliters of each sample was added to 225 mL of modified tryptone soya broth (mTSB) containing 30 g of TSB (Oxoid, UK), 1.5 g of bile salts no.3 (Oxoid, UK), 1.5 g of dipotassium phosphate, and 20 mg of novobiocin (Sigma Chemical Co., St. Louis, MO, USA) per liter. The inoculated broth was incubated at 41.5 $\pm$ 1°C for 18 - 24 h. After 6 h and 18 - 24 h, a loopful of the incubated broth was plated on CT-SMAC agar: Sorbitol Mac Conkey agar (SMAC; Oxoid) supplemented with cefixime and potassium tellurite (0.05 and 2.5 mg/L, respectively; CT supplement, Oxoid). After 18 - 24 h of incubation at 37 ± 1°C, non sorbitol fermenting colonies were selected and isolated. Presumptive colonies of Escherichia coli O157 were biochemically identified using API 20E (Bio Merieux, France). All biochemically identified non sorbitol fermenting colonies were subjected to slide agglutination with E. coli O157 latex test kit (Oxoid) (14).

III. Chemical Analysis

Titratable acidity (TA) (as lactic acid %) of raw milk and fresh cream was measured following the description by O'Connor (15).

IV. Statistical Analysis

SPSS pocket program for windows (version 16, 2007) was used for the statistical analysis. Paired samples T test was used for comparison of means. Values of different parameters were expressed as the mean ± standard error (SE).

Results

Table 1: Bacterial loads of raw milk and fresh cream samples (cfu/mL)

	Raw milk			Fresh cream		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean± SE
SPC*	2.1×10^4	4×10^8	$3.62 \times 10^{7a} \pm 1.37 \times 10^7$	1.5×10^4	7.3×10^8	$7.79 \times 10^{7a} \pm 2.80 \times 10^7$
Total coliforms	< 3	1.5×10^7	$1.65 \times 10^{6\,b} \pm 6 \times 10^5$	< 3	2.4×10^7	$4.21 \times 10^{6\,b} \pm 9.82 \times 10^5$
Faecal coliforms	< 3	7.5×10^6	$3.69 \times 10^{5\,b} \pm 2 \times 10^5$	< 3	2.4×10^7	$2.07 \times 10^{6\,b} \pm 7.64 \times 10^5$
E. coli	< 3	2.4×10^5	$2.83 \times 10^{4\,a} \pm 9.33 \times 10^3$	< 3	2.4×10^6	$1.89 \times 10^{5\,a} \pm 8.89 \times 10^4$
S. aureus	< 10	7×10^4	$4.68 \times 10^{3\,a} \pm 2.44 \times 10^3$	< 10	4×10^5	$3.5 \times 10^{4\,a} \pm 1.7 \times 10^4$

*SPC - standard plate count, [a] means p > 0.05, [b] means p < 0.05

Table 2: Bacterial loads of separators samples (cfu/mL)

	Min.	Max.	Mean	± SE.
Spc*	3.1×10^7	3.5×10^8	1.88×10^8	6.77×10^7
Total coliforms	2.4×10^7	> 1.1×10^8	8.85×10^7	2.15×10^7
Faecal coliforms	7.5×10^5	9.3×10^6	3.46×10^6	1.97×10^6
E. coli	< 3	2.1×10^5	1.06×10^5	6×10^4
S. aureus	< 10	1.6×10^4	4×10^3	4×10^3

*SPC - standard plate count

Table 3: Incidence of pathogens in examined samples

	No of samples	*E. coli*		*S. aureus*		*E. coli* O157	
		No	%	No	%	No	%
Milk	38	20	52.6	9	23.7	1	2.6
Cream	38	18	47.4	12	31.6	0	0
Separators	4	3	75	1	25	0	0

Table 4: Results of Titratable acidity of examined samples

	Minimum	Maximum	Mean ± SE
Raw milk	0.13	0.30	0.18 ± 0.01 [a]
Fresh cream	0.13	0.32	0.20 ± 0.01 [a]
Separators	0.20	0.31	0.24 ± 0.03

a means p > 0.05

The incidence of different microorganisms in raw milk and fresh cream is shown in Table 1. The SPC of raw milk and fresh cream ranged from 2.1 x 10^4 - 4 x 10^8 and 1.5 x 10^4 - 7.3 x 10^8 cfu/ml with a mean count of 3.62 x 10^7 ± 1.37 x 10^7 and 7.79 x 10^7 ± 2.8 x 10^7 cfu/ ml, respectively. Only 2.6 and 5.3 % of the examined raw milk and fresh cream samples were found to be in accordance with the <1 x 10^5 and < 3 x 10^4 cfu/ml set by Robinson (2002) for raw milk intended for further processing and fresh cream, respectively (Figure 1). Total coliforms and faecal coliforms were detected in 89.5 and 65.8 % of examined raw milk samples with a mean value of 1.65 x 10^6 ± 6 x10^5 and 3.69 x 10^5 ± 2 x10^5 MPN/ml and in 94.7 and 78.9 % of the examined fresh cream samples with a mean value of 4.21 x 10^6 ± 9.82 x 10^5 and 2.07 x 10^6 ± 7.64 x 10^5 MPN/ml, respectively (Table 1 and Figure 2, 3). The mean values for separators samples were 1.88 x 10^8 ± 6.77x 10^7 for SPC, 8.85 x 10^7 ± 2.15 × 10^7 for total coliforms, 3.46 x 10^6 ± 1.97 x 10^6 for faecal coliforms, 1.06 ×10^5 ± 6 x10^4 for *E. coli* and 4 x10^3 ± 4 ×10^3 cfu/ml for *S. aureus* (Table 2).

E. coli was isolated from 20 (52.6%) of 38 raw milk and 18 (47.3%) of 38 fresh cream samples with a mean count of 2.83 x 10^4 ± 9.33 x 10^3 and 1.89 x 10^5 ± 8.89x 10^4 cfu/ml, respectively . Nine (23.7%) of 38 raw milk samples and 12 (31.6%) of 38 fresh cream samples were contaminated with *S. aureus*, with an average of 4.68 x 10^3 ± 2.44 x 10^3 and 3.5 x 10^4 ± 1.7 x 10^4cfu/ml, respectively (Table 1, 3). *E. coli* and *S. aureus* were isolated form 75 and 25 % of the examined separators samples, respectively. E.coli O157 was isolated from one (2.6%) of 38 raw milk samples. None of the fresh cream and separators samples taken contained detectable levels of *E. coli* O157 (Table 3). Mean values of titratable acidity for raw milk, fresh cream and separators samples were 0. 18 ± 0.01, 0.20 ± 0.01 and 0.24 ± 0.03%, respectively (Table 4).

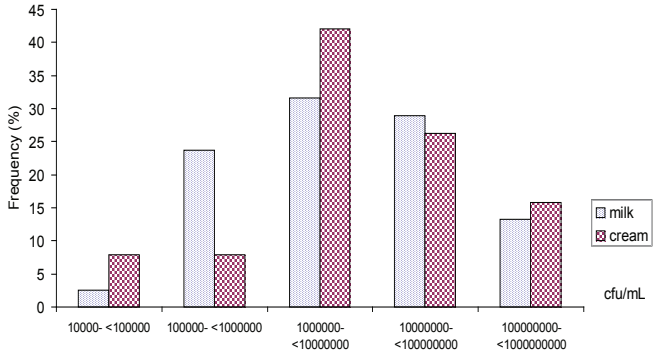

Figure 1: Frequency distribution of SPC in milk and cream samples

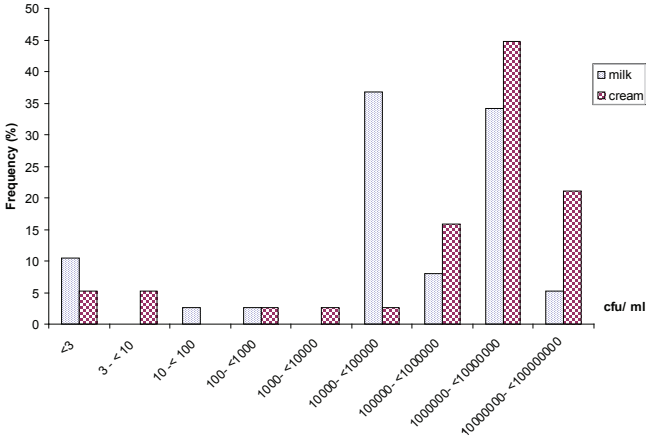

Figure 2: Frequency distribution of coliforms in milk and cream samples

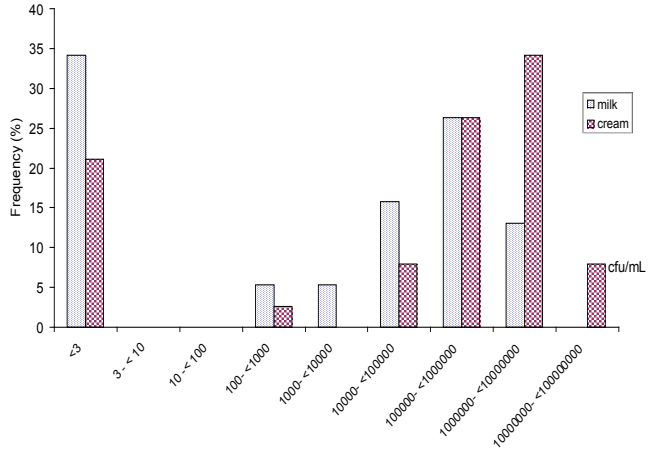

Figure 3: Frequency distribution of faecal coliforms in milk and cream samples

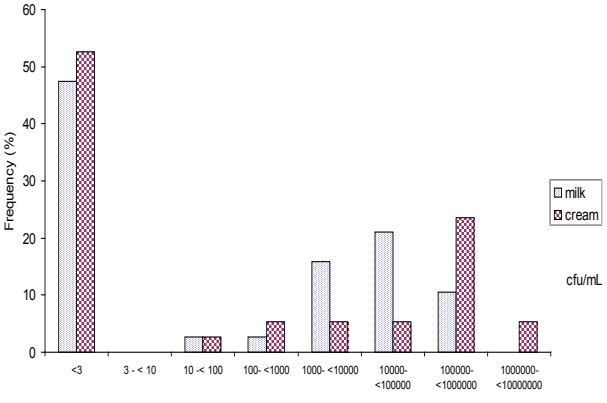

Figure 4: Frequency distribution of *E. coli* in milk and cream samples

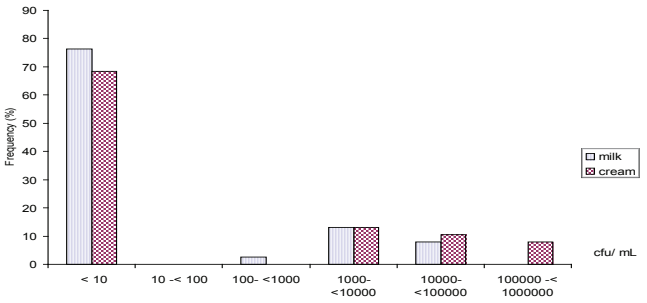

Figure 5: Frequency distribution of *S. aureus* in milk and cream samples

Discussion

The bacterial count in milk and cream potentially reveals the general conditions of sanitation and temperature control under which milk and cream were produced, handled and held.

Standard Plate Counts (SPC)

Raw milk contained an average SPC of 3.62 x 10^7 ± 1.37 x 10^7 cfu/ ml. Al-Tarazi et al. (17) and Korashy and Mohamed (9) reported considerably lower levels of SPC in raw milk with mean values of 1.1 x 10^7 and 4 x 10^6 cfu/ml, respectively. On the contrary, high levels of SPC in raw milk were reported by Moustafa et al. (5) and Sobeih et al (8) with mean values of 4 X 10^7 and 2.9 X 10^8 cfu/mL, respectively. The results found in those studies and present work indicate that raw milk and fresh cream are heavily contaminated. The mean count of SPC of fresh cream was marginally higher than those of raw milk but statistically not significant (P > 0.05). Possible reasons for the high counts could be due to infected udders of the cows, lack of knowledge about clean milk production, use of unclean equipment, poor personal hygiene, lack of cooling after milking and lack of heat treatment, which contribute to the poor hygienic quality of raw milk and fresh cream. Therefore, training and guidance should be given to the farmers in general milking hygienic practices and in keeping milk at low temperature to avoid microbial growth and lengthen the shelf life.

Coliforms, Faecal Coliforms and Escherichia coli

Many reports dealing with the occurrence of coliforms in raw milk have been accumulated. In those studies, various rates of coliforms were reported as 100, 100, 96, 88.7, 90, 41.3, 80 and 100% of examined raw milk samples by Saudi and Moawad (6), Ahmed and Sallam (18), Sobeih et al.(8), Al-Tarazi et al. (17), Chye et al. (19) , Korashy and Mohamed (9), Altalhi and Hassan (2) and El-Prince et al. (10), respectively. El-Essawy and Riad (20) and El-kosi (7) reported that all (100%) examined fresh cream samples were contaminated with coliforms. There are several reasons for these variations, such as differences in hygienic practices during milking, differences in geographic location and differences in seasonal trends. According to Robinson (16), total coliforms of raw milk intended for further processing should be < 500 cfu/mL and for fresh cream < 30 cfu/ mL. Thirty-two (84.2%) milk samples tested and 34 (89.5%) cream samples tested were found to be highly contaminated with coliforms over this limit (Figure 2).

The existence of coliforms may not necessarily indicate a direct faecal contamination of milk and cream, but is an indicator of poor hygiene and sanitary practices during milking and further handling, and presents potential hazard for people consuming such products. A significant difference occurred between the total coliforms of milk and total coliforms of cream (P< 0.05), suggesting that allowing milk samples temperature to resemble environmental temperature will favour the growth of different types of bacteria and could be responsible for high coliforms count in fresh cream.

E. coli and coliforms are often used as indicator microorganisms, and the presence of *E. coli* implies a risk that other enteric pathogens may be present in the sample. *E. coli* was isolated from 20 (52.6%) milk samples and 18 (47.3%) fresh cream samples (Table 3). All positive raw milk and fresh cream samples do not comply with Robinson (16) standards of ≤ 1 cfu *E. coli*/mL (Figure 4). The contamination rate in raw milk samples was extremely lower than the findings of Moustafa et al. (5), Sobeih et al. (8), Soomro et al. (21), Chye et al (19) and Altalhi and Hassan (2) as they found 66.6, 88, 65, 65 and 66% of their samples were contaminated by *E. coli*, respectively, but higher than the rate of 32, 27.5 and 3.3% reported by Ahmed and Sallam (22), Mezyed et al.(23) and El-Prince et al. (10), respectively. In previous studies, Ahmed and Sallam (22) and Mezyed et al. (23) reported that 38 and 15% of the cream samples tested were contaminated by *E. coli*, respectively.

Detection of *E. coli* in milk often reflects faecal contamination, although environmental coliforms have also been detected in milk. Milk can be easily contaminated by infected food handlers who practice poor personal hygiene or by water containing human discharges. Therefore, farmers must be educated in safe handling techniques and proper personal hygiene practices, including hand washing. Using potable water in dairy farm operations is crucial. Water must be safe and practically free from any type of bacterial contamination that may affect milk quality (19).

Staphylococcus aureus in raw milk and fresh cream

In the present study, 9 (23.7%) raw milk samples and 12 (31.6%) fresh cream samples were contaminated with *S. aureus* (Table 3). All positive samples were above the limits (100 cfu/mL) established by Robinson (16) standards (Figure 5). The isolation rate observed in this study was similar to those reported by Abdel-Hameed and El-Malt (24) who reported that 24 % of the examined milk samples were contaminated with *S. aureus*. However, Al-Tarazi et al. (17), El-Ziney and Al-Turki (25) and Guven et al. (26) reported higher levels of contamination for milk as they found 47, 70 and 33.3% of the milk samples were contaminated with *S. aureus*. *S. aureus* is frequently found in raw milk and milk products. Infections of the mammary gland (mastitis) represent a significant reservoir of toxigenic strains in raw milk. Storage of raw milk before separation under high environmental temperature permitting growth of *S. aureus* can stimulate the production of *S. aureus* enterotoxin. A significant difference did not occur between *S. aureus* count in milk and in cream ($P > 0.05$). This result highlights the unhygienic handling and inadequate personal hygiene.

S. aureus is one of the most common causes of food poisoning in humans worldwide. Although all raw milk and fresh cream samples have lower counts of *S. aureus* than 10^6 - 10^8 cfu/mL levels that are regarded as significant for human food poisoning to occur (27, 28**)**, they still present a public health hazard. Therefore, general hygienic practices aimed at minimizing bacterial contamination of milk and cream should be emphasized, as well as the growth of *S. aureus* must be prevented to avoid potential risk. Neither the absence of *S. aureus* nor the presence of small numbers of organism can provide complete assurance that the milk and cream are safe, since conditions inimical to the survival of *S. aureus* may result in a diminished population or death of viable microbial cells, while sufficient toxins remain to elicit symptoms of staphylococcal food poisoning (29).

Escherichia coli O157

E. coli O157 was isolated from one (2.6%) raw milk sample. None of the fresh cream and separators samples taken contained detectable levels of *E. coli* O157 (Table 3). There are a number of studies from Egypt and different countries concerning the incidence of *E. coli* O157 on raw milk and cream. Abdul-Raouf et al. (30), Abdel Khalek et al. (31) and Amer and Soliman (32) reported 6, 2 and 1% of raw milk examined in Egypt were contaminated with *E. coli* O157:H7, respectively. Allerberger and Dierich (33) reported 3% of the milk samples tested in Austria to be positive for *E. coli* O157:H7, Klie et al. (34) found that 3.9% of the raw milk analyzed in Germany was contaminated with *E. coli* O157:H7 and Chye et al. (19) detected *E. coli* O157:H7 in 33.5% of raw milk samples in Malaysia.

Although *E. coli* O157 was not detected in the cream and separators samples, the presence of other *E. coli* indicated that the potential modes of contamination by pathogenic *E. coli* are present during cream processing and handling. In previous survey, El-Kosi (7) reported that 20% of raw cream samples were contaminated with *E. coli* O157. This value is extremely higher than that obtained from this study.

Although the consumption of undercooked ground beef is still the traditional mode for *E. coli* O157:H7 infections, illnesses resulting from ingestion of contaminated raw milk are increasing. Clinical manifestations range from asymptomatic carriage through mild diarrhea to life-threatening conditions (35). The environmental niches for *E. coli* O157:H7 have not yet been clearly established. However, beef and dairy cattle appear to be a major reservoir for this pathogen (36). *E. coli* O157:H7 is apparently confined to the intestinal tract of dairy cattle and perhaps other animals as well. Therefore, preventing faecal material from contaminating the milk is an important step in reducing the prevalence of *E. coli* O157 and other pathogens in raw milk.

Chemical analysis

The mean values of titratable acidity for raw milk, fresh cream and separators samples were 0.18 ± 0.01, 0.20 ± 0.01 and 0.24 ± 0.03%, respectively (Table 4). Similar TA was reported in earlier studies on raw milk by Al-Zenki et al. (37) and Tasci (38) stating that the mean value of TA was 0.18%. On the other hand, higher values (0.199 and 0.23%) of TA for raw milk were reported by Al-Tarazi et al. (17) and Tassew and Seifu (39), while lower mean values (0.156 and 0.16%) of TA for raw milk were reported by Soler and Ponsell (40) and Korashy and Mohamed (9), respectively.

Fresh milk has a titratable acidity of 0.14 to 0.16% expressed as lactic acid(41) and loses its keeping quality when a critical acidity of 0.200 ± 0.01% is reached (37). However, 25 (65.79%) raw milk samples evaluated during the course of this study did not reach this critical value of acidity, thus still possessing good processing quality according to this quality parameter. A significant difference did not occur between TA of raw milk and fresh cream (P> 0.05). The increased acidity in raw milk and fresh cream may have been caused by keeping the milk at high room temperature (20 - 25°C) that favours the growth of lactic acid forming bacteria and other types of bacteria in the period between milk production and separation.

The use of inadequately cleaned and sanitized milking equipment is considered to be the major source of bacteria found in milk after its collection (42). The growth of a variety of microorganisms is supported by milk residues or remaining washing water left on milking equipment contact surface support. Bacteria multiply within these residues and contaminate milk passing through the equipment (43). In this study, according to the separators owner, separators were washed with water only, which could partly explain the high levels of bacterial contamination. According to the data portrayed in Table 2, it could be concluded that the separators had a definite effect on the cream quality.

Conclusions

Results of the study clearly indicate that bacteriological quality and safety of both raw milk and fresh cream produced by farmers were inferior. Separators are the main source of cream contamination and frequently the principal cause of high bacterial counts. High bacterial counts are likely to affect the keeping quality and safety of raw milk and fresh cream as well as products derived from it. The presence of pathogenic bacteria such *E. coli* O157 and *S. aureus* may pose a risk for public heath. Therefore, it is necessary to develop the hygienic status of locally produced raw milk and fresh cream, through educating the farmers in general hygienic practices and in handling their foods including correct storage to protect them from infection and to save a lot of products from deterioration. Also, information on health hazards associated with consumption of raw unpasteurized milk should be extended to the public, so that consumption of untreated raw milk and its products could be avoided.

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BAKTERIOLOŠKA KAKOVOST IN VARNOST SUROVEGA KRAVJEGA MLEKA IN SVEŽE SMETANE

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Povzetek: V raziskavi smo preučevali bakteriološko kakovost in prisotnost bakterij *S. aureus* in *E. coli* O157 v surovem mleku in sveži smetani. V zbiralnici mleka smo od junija do avgusta 2011 zbrali 80 vzorcev (38 vzorcev surovega mleka, 38 vzorcev sveže smetane in 4 vzorce iz ločevalnika). V vzorcih smo ugotavljali standardno število mikroorganizmov (SPC), skupne koliformne bakterije in koliformne bakterije fekalnega izvora (*E. coli* in *S. aureus*) ter patogene bakterije *E. coli* seva O157. Povprečne vrednosti CFU na ml mleka za SPC, skupne koliformne bakterije, koliformne bakterije fekalnega izvora, *E. coli* in *S. aureus*, so bile naslednje: 3,62 x 107, 1,65 x 106, 3,69 x 105, 2,83 x 104 in 4,68 x 103; na ml smetane pa: 7,79 x 107, 4,21 x 106, 2,07 x 106, 1,89 x 105 in 3,5 x 104. *E. coli* in *S. aureus* sta bila izolirana iz 52,6 % oziroma 23,7 % vzorcev surovega mleka, iz 47,4 % oziroma 31,6 % vzorcev smetane in iz 75 % oziroma 25 % vzorcev iz ločevalnika. E. coli O157 smo odkrili pri 2,6 % vzorcih surovega mleka. Srednje vrednosti kislosti v vzorcih surovega mleka, sveže smetane in ločevalnih vzorcih so bile 0,18 %, 0,20 % in 0,24 %. Visoke mikrobne vrednosti v vzorcih surovega mleka in sveže smetane lahko predstavljajo tveganje za zdravje potrošnikov in kažejo na nujnost izboljšanja higienskih standardov.

Ključne besede: mleko; smetana; *S. aureus; E. coli* O157; ločevalni vzorci