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PRESERVATION OF CAMEL MILK FOR SOMATIC CELL COUNT AND BACTERIOLOGICAL INVESTIGATION

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ABSTRACT

Effect of camel preservation milk on SCC and bacteriological isolation was investigated. Samples were collected from individual quarters (n=16) of 5 milking camels. Five minutes after receiving oxytocin (20 I.U), teat was washed and calf was released to stimulate the dam. Next, the suckling was interrupted, the teat was dried with tissue and the sample was collected aseptically for bacterial culture. Subsequently, milk samples were collected for SCC into tube with and without potassium dichromate. Frozen samples were thawed at room temperature and cultured one week after collection. For 4 days after milk samplings, SCC and culture of milk was conducted on a daily basis, maintaining the sample at 4°C. Out of 16 samples, 6 cases were free from any bacterial contamination and in 10 cases single pure bacteria were isolated. The possibility of isolating bacteria from fresh sample on the day of milk collection was greater than frozen specimen ($p=0.07$). SCC in milk samples without preservative decreased steadily during 4 days storage ($P<0.0001$); however, such decrease was not noticed in samples with preservative ($P>0.05$). Freezing adversely affected SCC with or without preservative. In conclusion, preservation of camel milk samples with potassium dichromate over 4 days at 4°C is a valid method to investigate SCC. However, due to the inconsistency in bacteriological isolation following storage at 4°C and freezing, it is advised to perform bacteriological investigation immediately following milk sampling in camel.

Key words: Bacterial isolation, camel milk, preservation, somatic cell count

In order to achieve meaningful and comparable results in somatic cell count (SCC) and bacteriological culture among different studies, it is important to follow standard procedures for storage of camel milk. It is well known that several factors could affect SCC in milk samples including the length and temperature of preservation, freshness of sample, the type of preservatives and cell counter used (Sanchez *et al,* 2005; Gonzalo *et al,* 2003; Martinez *et al,* 2003; Barkema *et al,* 1997). Significant decline in the SCC occurred following prolonged preservation of goat (Kennedy, 1982; Sanchez, 2005), ewe (Gonzalo 2003) and cow (Vermont 1995) milk at ambient temperature. Smaller decrease in SCC occurred following freezing of ewe (Gonzalo *et al,* 2003; Martinez *et al,* 2003) and cow (Barkema *et al,* 1997) milk samples. This could be due to nuclear degeneration and cell injury after freezing (Read *et al,* 1964; Barkema *et al,* 1997). SCC of milk samples preserved with bronopol or potassium dichromate was not affected by freezing in ewe (Martinez *et al,* 2003) and goat (Sanchez *et al,* 2005).

Cow milk samples can be preserved at very low temperature (-80°C) for 14 days without any changes in the cultural results of bacteriology (Bashandi and Heidar, 1979). False negatives for *E.coli* and *Arcanobacterium pyogenes* and false positives for Coagulase Negative Staphylococci (CNS) increased in cow milk samples following freezing at -20°C for 4, 8 and 16 weeks (Schukken *et al,* 1989). Freezing milk samples at -20°C for 6 weeks did not affect the number of *Staphylococcus* spp., *Corynebacterium bovis* or E.coli (Murdogh *et al,* 1996; Schukken *et al,* 1989). Interestingly, after freezing cow milk samples for 23 days, the possibility of isolating *Staphylococcus aureus* increased for 1.48 times (Villanueva *et al*, 1991). There is no guideline to store camel milk samples in order to perform somatic cell count (SCC) and bacteriological investigation. The purpose of this study was to

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investigate the effect of length of preservation at 4°C, preservative and freezing on SCC and bacteriological isolation.

Materials and Methods

Experimental Design

Dromedary milking camel (n=5), 7-11 years of age, 2-4 months after calving, with the average daily milk production of 6 kg were used in this study. These were milked trice daily (5:00, 16:00, 21:00) and maintained on pasture throughout the day.

Five minutes prior to milking, camel received oxytocin, the teat was washed and the camel calf released to stimulate the dam. CMT was used according to the procedure described previously (Schalm *et al*. 1971). Before milk sampling, the teats were disinfected with cotton moistened with 70% alcohol. After discarding the first few squirts of milk, about 50 ml of milk were collected into sterile bottle for bacteriological examination and about 250 ml of milk were collected for SCC. Samples were kept at 4°C and carried to the laboratory within 8 hr. In the laboratory, out of 250 mls collected samples, 25 mls with and without potassium dichromate (Floka, Boches, Switzerland) were frozen at -20°C. Under sterile condition, bacteriological samples were distributed into 2 sterile tubes. One tube was frozen in a commercial freezer at -20°C and one tube was stored at 4°C. Frozen samples were thawed after one week at room temperature and assayed for SCC and bacteriology. Samples which stored at 4°C were assayed for SCC and bacteriology on a daily basis (Days 0= Day of milk collection) for 4 days.

Bacteriological Culture

Bacteriological isolation was carried out according to standard procedure (Quinn *et al,* 1994). In brief, milk sample (30-50 µL) was streaked on 5% sheep blood agar (Cat: 10886; Merck, Germany) and MacConkey agar (Cat: 5465; Merck, Germany) plates. Inoculated plates were incubated at 37°C for 5 days. Presumptive identification of bacteria on primary culture was done on the basis of colony morphology, haemolytic characteristics, Gram-stain and catalase test. Briefly, Staphylococci were identified based on coagulase test. Streptococci isolates were evaluated based on CAMP reaction, aesculin hydrolysis test and growth on 6.5% sodium chloride. Gram-negative isolates were further tested using triple sugar iron (TSI), urease test and indol test. Further standard biochemical tests were also carried out to identify species of the bacteria.

Somatic Cell Count

Somatic cells were counted using Fossomatic machine (Fossomatic 5000, Fossomatic Company, Denmark). Prior to SCC, standard sample, consisting 389000 cells, was used to calibrate the machine. Samples of 25 ml volume were assigned into special racks and allowed to be homogenized and counted individually by the detector.

Statistical Analysis

The pattern of changes in SCC within groups was analyzed using GLM procedure including repeated measures in the model using SAS (SAS, 2007). The frequency of observations was analyzed using Goodness-of-fit test. Data were presented as mean ± SEM or percentage.

Results

From 16 milk samples collected from lactating camel of Turkmen county, no bacteria was found in 6 cases and pure bacteria was isolated in 10 other quarters. The isolated bacteria included *Staphylococcus schleifer* (n=5), *Staphylococcus saprophyticus* (n=3), *Staphylococcus epidermicus* (n=1) and *Streptococcus uberis* (n=1; Table 1). There was no relationship between CMT scores or SCC and possibility of isolating bacteria. Accordingly, no bacteria were isolated in 1 (98±3 SCC), 1 (194±5 SCC) and 2 (955±20 and 968±19 SCC) cases with T, 1 and 2 CMT scores. On the contrary, in 1 case with CMT score of 0 (46±2 SCC) a single pure bacterium was isolated (Table 1). Excluding one quarter in which a single pure bacterium was isolated consistently throughout daily culture of fresh sample and frozen sample, in all other samples, the consistent isolation of bacteria was not achieved (Table 1). The frequency of bacterial isolation on days 0, 1, 2, 3 and 4 of fresh samples and frozen sample were 7, 6, 6, 5, 6 and 3 (Table 1). There tend to be a significant difference between frequency of bacterial isolation on day 0 and frozen sample ($P=0.07$; Table 1). There was significant reduction in SCC from day 0 to 4 in fresh milk samples that did not receive potassium dichromate ($P<0.0001$; Figure 1). However, there was no difference among daily milk samples for fresh milk with potassium dichromate ($P>0.05$; Fig 1). SCC was reduced significantly in frozen samples both with (1018±293) and without (1097±332) preservative compared to fresh samples ($P<0.01$).

Discussion

The objective of this study was to investigate the effect of preservation (length, presence of preservative

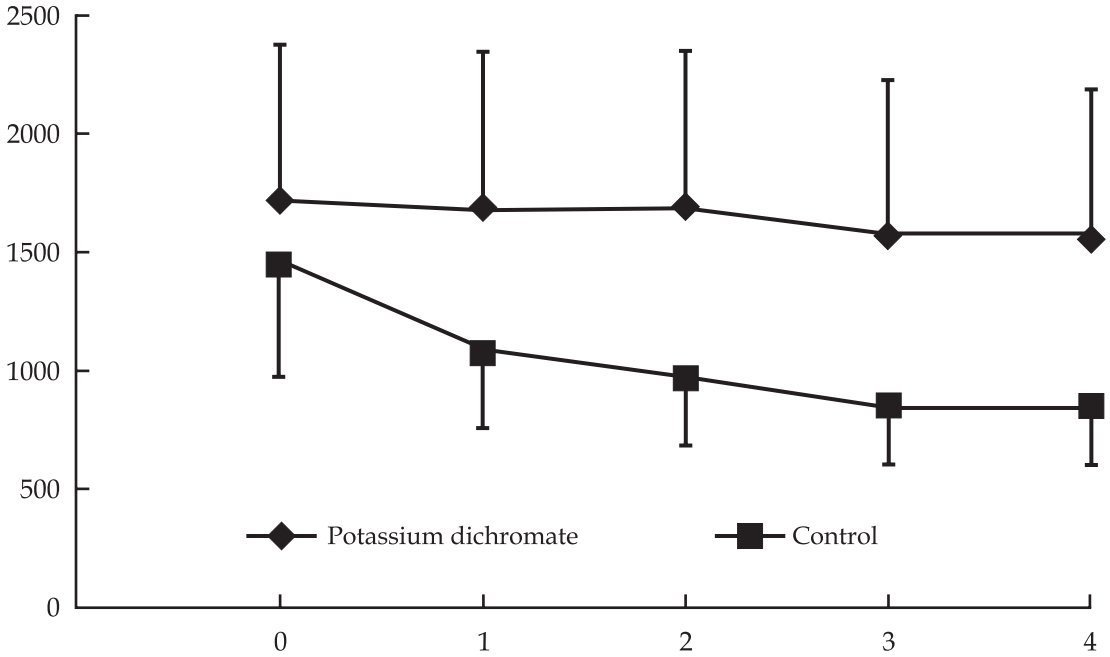

Fig 1. Somatic Cell Counts from milk samples of individual quarters of dromedary camels that were maintained at 4˚C for 4 days with or without potassium dichromate.

and freezing) on SCC and bacteriological isolation of camel milk. The results have shown the possibility of preserving camel milk at 4ºC in the presence of potassium dichromate for at least 4 days. In contrast, without preservative, there was a significant decline in SCC at the same conditions. Increasing the length of milk preservation in the presence of preservative could decline SCC in goat milk after 10 days (Sanchez *et al,* 2005), in ewe milk after 3 days (Gonzalo *et al,* 2003) and in cow milk after 42 days (Vermont *et al,* 1995).

In the present study, the inconsistency in isolating bacteria was observed during daily culture of the same specimen. This may be due to the time interval between sampling and culture of the specimen, during which pathogens may be eliminated because of anti-bacterial factors available in camel milk (Salami *et al,* 2010; El-Hatmi *et al,* 2007; Korhonen and Pihlanto, 2006). Problems in associations with small number or unequal dispersion of bacteria in the sample as occurred during isolation of *Salmonella enteridis* in egg (Carrique-Mas and Davies, 2008) may also be considered as another possible factor for inconsistency in isolating bacteria in camel milk sample.

The chance of isolating bacteria tended to be greater in fresh samples, particularly on the day of milk sample collection than the frozen samples. This, in turns, indicates that camel milk sample may be better to be cultured immediately after collection in order to increase the chance of isolating bacteria. In the present study, the milk samples were frozen at -20ºC within 3 hours after collection. The temperature, at which the milk is frozen, and type of bacteria are considered as the main two factors contribute to increase the chance of isolating bacteria after thawing. Cow milk samples can be preserved at very low temperature (-80ºC) for 14 days without any changes in the cultural results of bacteriology (Bashandi and Heider, 1979). This method could also be used for diagnosis of Gram Negative Bacilli in goat milk samples as well (Sanchez *et al,* 2003). There is a decrease in the chance of isolating *E.coli* and *Arcanobacterium pyogenes* and an increase in the chance of isolating Coagulase Negative Staphylococci after freezing at -20º (Schukken *et al,* 1989). Freezing milk samples at -20ºC for 6 weeks did not affect the recovery of *Staphylococcus* spp., *Stereptococcus* spp., *Corynebacterium bovis* or *E.coli* (Murdogh *et al,* 1996). Interestingly, freezing milk samples for 23 days, increased the chance of isolating *Staphylococcus aureus* for 1.48 times (Villanueva *et al,*1991).

In some cases (n= 5 quarters) with CMT scores of T, 1 and 2 in association with relatively high SCC, no bacteria was isolated. Similar results were reported in almost 30 per cent of cow milk samples (Philpot and Nikerson, 2000). In special occasions, when SCC is relatively high, pathogens may not be isolated. Such incidence may occur during the recovery of udder when the infection was resolved but somatic cells still remained elevated throughout the healing process (Philpot and Nikerson, 2000). Other factors

Table 1. Bacteriological isolation from fresh and frozen quarter milk samples of dromedary camels. Data were presented as mean±SEM.

Camel	Quarter	CMT	SCC (x1000)	Day of Preservation of Fresh Milk Sample at 4°C					
				0	1	2	3	4	Frozen
10	RF	0	24±2	negative	negative	Negative	Negative	negative	negative
11	RF	0	46±2	negative	negative	Negative	*Staph. saprophyticus*	negative	*Staph. saprophyticus*
16	LB	T	98±3	negative	negative	Negative	Negative	negative	negative
11	LF	1	135±6	*Staph. epidermidis*	*Staph. epidermidis*	*Staph. epidermidis*	*Staph. epidermidis*	*Staph. epidermidis*	negative
16	LF	1	194±5	negative	negative	Negative	Negative	negative	negative
19	RF	1	314±7	*Staph. schleifer*	negative	*Staph. Schleifer*	Negative	negative	negative
16	RF	2	582±25	negative	negative	Negative	*Staph. saprophyticus*	*Staph. saprophyticus*	negative
16	RB	2	680±19	negative	negative	*Staph. schleifer*	Negative	*Staph. schleifer*	negative
19	LF	2	955±20	negative	negative	negative	Negative	negative	negative
17	LB	2	968±19	negative	negative	negative	Negative	negative	negative
19	RB	2	1415±18	*Staph. schleifer*	*Staph. schleifer*	*Staph. schleifer*	Negative	*Staph. schleifer*	negative
17	LF	2	1735±33	negative	negative	negative	Negative	negative	negative
17	RB	2	1793±55	*Staph. schleifer*	*Staph. schleifer*	negative	*Staph. schleifer*	*Staph. schleifer*	*Staph. schleifer*
19	LB	3	2199±74	*Staph. schleifer*	*Staph. schleifer*	*Staph. schleifer*	negative	negative	negative
17	RF	3	4783±383	*Staph. saprophyticus*	*Staph. saprophyticus*	*Staph. saprophyticus*	*Staph. saprophyticus*	*Staph. saprophyticus*	*Staph. saprophyticus*
10	LF	3	9654±540	*Strep. uberis*	*Strep. uberis*	negative	negative	negative	negative

such as increased age, advanced stage of lactation, season, stress and trauma also involved in increasing SCC in the absence of pathogenic organisms (Dohoo and Meek, 1982; Schultz, 1977). The lactation effect may be due to the sloughed epithelial cells, whereas increased age reflects a higher incidence of infection following repeated exposure and recovery (Schultz, 1977; Suheir *et al,* 2005).

Although freezing milk sample could facilitate the process of sampling for control and prevention of mastitis, the results of this study indicated that freezing not only affected bacteriological investigation but also it decreased SCC. This is in agreement with previous reports in ewe and cow in which SCC decreased following freezing of milk samples (Gonzalo *et al,* 2003; Martinez *et al,* 2003; Barkema *et al,* 1997). This could be due to the nuclear degeneration and cell injury after freezing (Read *et al,* 1964; Barkema *et al,* 1997). SCC of milk samples preserved with bronopol or potassium dichromate was not affected by freezing in ewe (Martinez *et al,* 2003) and goat (Sanchez *et al,* 2005). In the present study, freezing camel milk samples in the presence of potassium dichromate declined SCC.

In conclusion, it is possible to preserve camel milk for SCC in the presence of potassium dichromate at 4°C for 4 days. It is advised to perform bacteriological investigation on camel milk immediately after milk sampling. Freezing camel milk at -20°C and thawing at room temperature is not recommended due to the significant decrease in SCC and reduction in the possibility of isolating bacteria.

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