Vol 10 No 2, p 173-178

MICROBIOLOGICAL STUDIES ON CAMEL MILK IN NORTH SINAI, EGYPT

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ABSTRACT

Fifty raw camel milk samples were randomly collected under sterile condition from different camel herds at El-Zaranik and El-Arish cities, North Sinai Governorate, Egypt. Fifteen of the examined samples were positive for Aerobic spore former count, 7 samples were positive for total coliform count and 11 were positive for total *enterococcal* count. *Pseudomonas, Aeromonas,* coagulase positive *Staphylococcus aureus* were counted. *Salmonella, Listeria* and *Yersinia* species could not be isolated in all examined samples. Many fungal species were isolated from the examined milk samples including *Aspergillus*, *Penicillium*, *Alternaria*, *Acremonium* and *Chrysosporium* species. The lipolytic activity of the isolated mold was examined. Among ninty fungal strains tested, seventy isolates were positive with varying degree. The economical and public health importance of the isolated microorganism as well as control measures for improving the milk quality was discussed.

Keywords : Camel, Egypt, microbiological tests, milk

Microorganisms either through their presence and/or multiplication can affect the milk quality, therefore much attention has been given to produce milk with minimal microbial contamination (Robinson, 1990). Microbial contaminants may cause serious health problems also. Several methodologies are used to evaluate the bacterial quality of milk, the standard plate count is the most common. The second test that became prevalent in the recent years is the preliminary or pre-incubated (PI) bacterial count. This test provides better estimate on psychrophilic bacteria and the on level farm sanitation. Although psychrophilic bacteria are usually non pathogenic but they could attack milk protein and fat leading to milk spoilage (Stevenson and Rowe, 1994 and Agrawal *et al*, 2002).

The battle of milk spoilage is assisted by new information on enzyme production by spoilage fungi. Tainting of milk and milk products may be due to production of lipase and protease enzymes resulting off-flavours described as rancid, soapy, unclean and bitter. As the camel milk is rich in specific protease inhibitors the lipase enzyme is considered as the main factor affecting milk spoilage (Braun *et al*, 2002 and Saxena *et al*, 2003).

As desert nomads drink milk immediately after milking in the raw state, therefore present study was planned to evaluate the microbiological quality of camel milk and testing for lipase enzyme produced by isolated mold strains that could affect milk quality.

Materials and Methods

Collection of samples

Fifty random camel milk samples (150 ml each) were collected from different camel herd at El-Zaranik and El-Arish, North Sinai Governorate. The milk samples were kept at 4°C in ice box until analysed within 24 hours. One millilitre of milk was transferred to a sterile tube containing 9 ml sterile Ringer solution as a diluent from which ten fold serial dilutions were prepared.

Organoleptic examination

The collected milk samples were sensory scored using score cards for flavour and odour (45 points), appearance and colour (20 points) and bacteria (35 points). The scores were averaged by five panelists according to Nelson and Trout (1981).

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Microbiological analysis

1. Total colony count: The samples were plated onto standard plate count agar media and incubated at 10°C for 7 days, 37°C for 48 hours and 55°C for 24 hours for psychrophilic, mesophilic and thermophilic count, respectively.
2. Aerobic spore former count according to A.P.H.A (1992): Ten ml of milk samples were heated in water bath at 80°C for 10 minutes after which it was left to cool at room temperature and then 0.1 ml from each dilution was inoculated onto duplicate plates of dextrose tryptone agar media using spreading techniques and incubated at 37°C for 48 hours.
3. Coliform count (MPN/ml) using three tubes employing lauryl sulphate broth containing durhams tube according to A.P.H.A. (1992).
4. Enterococcal count by plating onto enterococci selective media according to ICMSF (1996).
5. Pseudomonas and Aeromonas count by surface plating onto glutamate starch phenol red agar containing 1 lac i.u. penicillin G/L and incubated at 25°C for 72 hours as recommended by Collins *et al* (1995).
6. *Staphylococcal* count (coagulase positive) by plating the sample onto Baired parker agar media with 50 ml egg yolk suspension in potassium tellurite and incubated at 37°C for 48 hours characteristic colonies appeared as black, smooth with white edges and opaque zone were subcultured in brain heart broth and confirmed by assaying coagulase positive with rabbit plasma according to Nathalie and Gueguen (1997).

Isolation and identification of some pathogenic microorganisms

Isolation of *Salmonella* by inoculation of milk sample firstly in preenriched media, selenite broth incubated at 37°C for 18 hours then onto Rappaport - Vassiliadis broth at 37°C for 18h. Loopful were streaked on xylose lysine desoxycholate (XLD) media according to D'Aust (1991).

Isolation and identification of *Listeria* was done according to Fedio and Jackson (1992). Milk samples were preenriched in half fraser broth incubated at 37°C for 18 hours followed by enrichment on fraiser broth incubated at 37°C for 18 hours. Loopful was streaked onto oxford palcam media incubated at 37°C for 24 hours.

Isolation of *Yersinea enterocolitica* was done according to Larkin *et al* (1991). Milk samples were enriched in peptone water broth containing sorbitol and bile salt at 25°C for 5 days then plated onto cefsulodin irgasan novobiocin agar media and incubated at 30°C for 24 hours.

7. Isolation and identification of mold and yeast: Total mold and yeast count were perfomed by inoculation of milk sample onto sabouraud dextrose agar media containing 0.05mg chloramphenicol/ml incubated at 25°C for 7 days. Isolated molds were subjected for identification according to their morphological and microscopical characters according to Pitt and Hocking (1997).
8. Detection of lipolytic activity of the isolated molds was done according to Setala and Garanina (1986) and Hankin and Anagnostakis (1995).

The basal media was composed of peptone1%, magnesium sulphate 0.2%, calcium chloride 0.02%, tween 80 1% and agar 1.5% at pH 6. The media was autoclaved and sterilised at 121°C for 15 min. Tween 80 was separately autoclaved and then added to the media. Duplicate plates were inoculated by spot inoculum of the isolated mold strain and incubated at 25°C for 7 days. Opaque zone surround mold growth indicate positive lipolytic reaction.

Results and Discussion

Camel milk is considered as an important part of the life of desert dwellers providing them a source of protein and energy. Physical and microbiological characters are the main factors, which measure the milk quality. The organoleptic properties of camel milk samples are shown in (Table 1). All the samples were generally opaque-white in colour with sweet pleasant taste. The flavour and odour, appearance and colour and bacteria scored 42 ± 0.03, 16 ± 0.12 and 28 ± 0.40 points, respectively. The examined camel milk samples were good and within the acceptable level. Nearly similar findings were reported by Sawaya *et al* (1984); Mohamed, (1990); Abu-Leiha (1989); Elamin and

Wolcox (1992) and Farah (1993). The type of feed and the availability of drinking water may affect the physical properties of camel milk (Wilson, 1997 and Landis, 2003).

The results given in table 2 revealed that total psychrophilic, mesophilic and thermophilic count were detected in 30, 60 and 10% of the samples with an average values $3.5x10^3$, $2.1x10^4$ and $4.4x10^2$ (cfu/ml), respectively. The samples contain higher mesophilic occurrence and number than psychrophilic and thermophilic count. Higher results were recorded by Al-Mohizea (1986) and Sallam and Nagah (1993). This might be due to the physical environmental condition of desert, which allowed most of milk flora and pathogenic bacteria to grow at 37°C (Varnam and Sutherland, 1994 and Ray, 1996). There is no Egyptian standard for raw camel milk. However, total colony count not to exceed 50000 cfu/ml is widely accepted standard for bovine milk (Boor and Murphy, 2002). Therefore comparing our results with the standard, most of the examined samples proved to be satisfactory.

Table 1. Organoleptic properties of camel milk samples.

Criteria	Score	± S.E.M.
Flavour and odour (45)	42	0.03
Colour and appearance (20)	16	0.12
Bacteria (35)	28	0.40
Total %	86	0.09

Table 2. Total colony count in the examined raw camel milk (cfu/ml) (N* = 50).

Total colony	No. positive	%	Min	Max	Mean	± S.E.M
PC	10	30	50	$9x10^4$	$3.5x10^3$	$1.1x10^2$
MC	30	60	40	$18x10^6$	$2.1x10^4$	$1.9x10^3$
TC	5	10	20	$5x10^4$	$4.4x10^2$	$1.3x10^2$

* = No. of samples; PC = Psychrophilic count, MC = Mesophilic count, TC = Thermophilic count

Results shown in table 3 demonstrate that aerobic spore forming bacteria were isolated from 30% of the examined camel milk samples with a mean value of $8.8x10^3 \pm 1x10^2$ cfu/ml. Higher count were reported by Al-Mohizea (1986) and Sallam and Nagah (1993). Aerobic spore forming bacteria are widely distributed in nature and may gain access to milk through various routes including air, water and utensils (John and Despencer, 2001).

The data recorded in table 3 revealed that coliform were presented in 14% of the examined camel milk samples with an average $9.5x10^4 \pm 4.6x10^3$ cfu/ml. In general, coliform count may be used as an indication of sanitation. The presence of more than 100 coliforms/ml indicate bad hygienic measure during milk production, handling and distribution. Moreover, about 30% of people in industrial countries and hundred of millions of people in developing countries suffer from diarroheal disease (World Health Organisation, 2000). Moreover, contamination of milk with coliform might induce many changes leading to economic losses (Robinson, 1990).

The *Enterococcal* count were existed in 22% of the examined camel milk samples with a mean value of $3.2x10^3 \pm 1.4x10^2$ cfu/ml. They are normal inhabitants of the alimentary tract of man and animal and their presence indicates faecal contamination. Also sometimes food poisoning may occur particularly if milk is heavily contaminated (Ray, 1996).

It is evident from the results presented in table 3 that *Pseudomonas* and *Aeromonas* count could be detected in 18% of the examined camel milk samples with a mean value of $2.2x10^3 \pm 1x10^2$ cfu/ml. Pseudomonas species are found predominately in soil and water. They are commonly associated with milk spoilage through production of heat stable enzymes, protease and lipase inducing off-flavour and shorten shelf life time of milk (Celestino *et al*, 1996).

Coagulase positive *Staphylococcus* was isolated from 10% of the samples with a mean value of $6.6x10^3 \pm 3.5x10^2$ cfu/ml. These results agreed to certain extent with those reported by Barbour *et al* (1985) and Sallam and Nagah, (1993). It is potenial pathogen causing mastitis and has been implicated in many food borne intoxications associated with consumption of raw milk (Mostafa *et al*, 1987).

Camel milk is usually consumed in raw state by nomade. It is therefore of interest to know the activity of natural antimicrobial protein. The ability of camel milk to inhibit the growth of pathogenic bacteria through the high lysozymes, lactoperoxidase, immunoglobulin and N-acetyl

Table 3. Microbiological examination of camel milk samples (cfu/ml) (N* = 50).

Tests	No.	%	Min	Max	Mean	± S.E.M.
Aerobic spore former	15	30	10	6×10^5	8.8×10^3	1×10^2
Coliform count	7	14	3.6×10^2	8.2×10^6	9.5×10^4	4.6×10^3
Total EnterococcaJ count	11	22	60	4.4×10^5	3.2×10^3	1.4×10^2
Pseudomonas and Aeromonas	9	18	10	9.4×10^4	2.2×10^3	1×10^2
Stapylococcus count	5	10	20	$7.1 \times 1\ 0^4$	6.6×10^3	3.5×10^2
Total mold and yeast count	50	100	100	5.7×10^8	6×10^6	2.9×10^5

* = No. of samples

glucosaminidase (NAGase) have been reported by several authors (Al-Nakli, 1984; Sallam, 1991 and Farah, 1993). This may explain why *Salmonella*, *Listeria* and *Yersinea enterocolitica* could not be recovered in all the examined camel milk samples.

Mold and yeast were isolated from all samples with a mean value of $6x10^6 \pm 2.9x10^5$ cfu/ml. *Genus Aspergillus*, *Penicillium*, *Acremonium*, *Alternaria* and *Chrysosporum* could be isolated at percentages of 62, 36, 32, 28 and 22%, respectively. Ninety mold isolates were tested for their lipolytic activity. Seventy strains could produce lipase enzyme which hydrolysed the fatty compound tween 80 (poly exoethylene sorbitan monooleated) forming oleic acid precipitated as calcium oleate. Lipase production differs not only among the different mold species but also among the isolates in the same species (Tables 3 and 4).

Table 4. Incidence of molds and their lipolytic activity in the examined camel milk samples (N* = 50).

Isolated strains	No.	%	Lipolytic activity	
			positive	%
Genus Aspergillus	31	62	20	64.5
A. flavus Link	14	28	10	32.3
A. fumigatus Fresenius	11	22	6	19.3
A. sydowii (Bain and Start) Thom	5	10	4	12.9
A. ustus (Bain and Start) Thom	1	2	0	0.0
Genus Penicillium	18	36	16	88.9
P. aurantiogriseum Dierckx	9	18	8	44.4
P. funiculosum Thom	5	10	4	22.2
P. purpurogenum Stoll	3	6	3	16.7
P. viridicatum Westling	1	2	1	5.6
Genus: Acremonium	16	32	9	56.3
A. fusidiodes w. Gams	10	20	5	31.3
A. strictum w. Gams	6	12	4	25.0
Genus Alternaria	14	28	14	0
A. alternata Keissler	9	18	9	0
A. tenuissima (Kunze ex Peris) Wilt	5	10	5	0
Genus Chrysosporum	11	22	11	100
C. carmichilli van oorschot	7	14	7	63.6
C. keratinophilium Frey carmichael	4	8	4	36.4

Salmonella, Listeria and *Yersinea enterocolitica* could not be detected in any of the examined camel milk samples.

* = No. of samples

Generally, an analysis of microflora of camel milk showed that there are many saprophytic fungi inhabiting camel milk. The high incidence of the fungi may be attributed to their widespread distribution in nature as well as their high ability to adopt at wide range of environmental conditions. They may also constitute part of normal flora of camel wool (Bagy and Abdel - Hafez, 1985 and Laila Nasser *et al*, 1998). The presence of large number of molds in camel milk could lead to a public health hazard or may be responsible for undesirable changes and inferior quality of the milk specially if processed. By growth and metabolic activity lipolytic mold may cause spoilage through production of lipase enzyme leading to off flavour (Sawaya *et al*, 1984; Mohamed, 1990; Hubbert *et al*, 1996 and Conesa *et al*, 2001). Lipase enzyme catalyses the hydrolysis of triglycerides of fatty acid yielding mono-glycerides, diglycerides, glycerol and free fatty acids. Such enzyme may predict a defect during long storage and processing (Günther *et al*, 2001 and Layer and Keller, 2003).

The high level of lipase enzyme may be responsible for temporal bitterness noted in some soft cheese manufactured from camel milk (Farah, 1993; Abu-Tarboush *et al*, 1998; Giardet *et al*, 2000; Attia *et al*, 2001).

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