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HYGIENIC QUALITY OF EGYPTIAN CAMEL MILK

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ABSTRACT

Fifty raw Egyptian camel milk samples were randomly collected under sterile condition from different camel herds at El-wahat El -baharia, Giza Governorate, Egypt. Thirty per cent of the examined samples were positive for *Aerobic spore former* count, 24% were positive for total *coliform* count and 20% were positive for total *enterococcal* count. *Pseudomonas, Aeromonas,* coagulase positive *staphylococcus aureus* were counted. *Salmonella, Listeria, and Yersinia* species could not be isolated. Many fungal species were isolated from including *Aspergillus, Penicillium, Alternaria, Acremonium* and *Chrysosporium* species. The lipolytic activity and aflatoxin production of the isolated molds were examined. Among 90 fungal stains tested, 70 were positive for lipolytic activity with varying degree while none of the screened *A. flavus* and *A. parasiticus* strains were aflatoxin B1, B2, G1 and G2 producers. The economical and public health importance of the isolated microorganisms as well as control measures for improving the milk quality were discussed.

Key words: Camel, hygienic quality, milk

Camel milk is an important nutritional food in areas where protein sources are not readily available like in Africa and growing countries. It may be used alone as single food for children and elderly people. It is highly nutrious and delicious, low in fat and cholesterol while rich in anti-protease inhibitors, vitamin C, insulin, potassium and iron (Kappeler *et al*, 1998 and Tefera and Gebreah, 2004).

The use of camel milk for medicinal purposes is a recent exciting development, where it proved to have an important role in the treatment of malnutrition, jundice, anaemia, diabetes, asthma, ulcers, milk allergy, lactase deficiency and breast cancer (Agarwal *et al*, 2002; Shiller, 1990; Elsayed *et al*, 1992; Gorban and Izzeldin, 2001; and Guliye *et al*, 2002).

Camel milk is considered as favorable medium for multiplication of microorganisms. Microbial contaminants reduce the milk quality and cause serious health problems. Several methodologies are used to evaluate the bacterial quality of milk and 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 on the farm level 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).

Tainting of milk and milk products may be due to production of lipase and protease enzymes from fungi resulting into off-flavours described as rancid, soapy, unclean and bitterness. As camel milk is rich in specific protease inhibitors so only lipase enzyme is considered as the main factor affecting milk spoilage (Braun *et al*, 2002 and Saxena *et al*, 2003).

As desert nomads drink camel milk immediately after milking in the raw state, therefore, it was planned to evaluate the microbiological quality of milk and its testing for lipase enzyme and aflatoxin B1, B2, G1 and G2 production by isolated mold strains that could affect camel milk quality.

Materials and Methods

Collection of samples

Fifty random camel milk samples (150 ml each) were collected from different camel herds at El-wahat El–baharia, Giza Governorate, Egypt. 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.

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). All collected milk samples were examined for titratable acidity

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(TA) expressed as lactic acid % using 0.1 sodium hydroxide and 1% phenolphthalin solution in 95% ethanol as endpoint indicator according to Marshall (1992). The pH of milk samples was determined using pH meter (model 920 Orion Inc., Boston MA). Resazurine reduction test was carried out according to the method reported by Chalmers (1962).

Microbiological analysis

·· Microbial counts

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 h and 55°C for 24 h for psychrophilic, mesophilic and thermophilic count, respectively according to Anonymous (1996).

2. Aerobic spore former count

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.

From each dilution 0.1 ml was inoculated onto duplicate plates of dextrose tryptone agar media using spreading techniques and incubated at 37°C for 48 h according to Anonymous (1992).

3. Coliform count (MPN/ml) using three tubes employing lauryl sulphate broth containing durhams tube according to Anonymous (1992).

4. Enterococcal count by plating onto enterococci selective media according to Anonymous (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 h 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 h. 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 h then inoculated onto Rappaport-Vassiliadis broth at 37°C for 18 h. Loopful inoculum was streaked on xylose lysine desoxycholate(XLD) media according to D'Aust (1991).

1. Isolation and identification of *Listeria* according to Fedio and Jackson (1992).

Milk samples were preenriched in half fraser broth incubated at 37°C for 18h followed by enrichment on fraiser broth incubated at 37°C for 18h. Loopful inoculum was streaked onto Oxford palcam media incubated at 37°C for 24 h.

2. Isolation of *Yersinea enterocolitica* 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 24h.

3.Isolation and identification of *mold* and *yeast*

Total *mold* and *yeast* counts were perfomed by inoculation of milk sample onto Sabouraud dextrose agar media containing 0.05 mg chloramphenicol/ ml incubated at 25°C for 7days. Isolated molds were subjected for identification according to their morphological and microscopical characters according to Pitt and Hocking (1997).

·· Detection of lipolytic activity of the isolated molds according to Setala and Garanina (1986) and Seeley *et al* (1991).

The basal media was composed of peptone 1%, 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 surrounded by mold growth indicated positive lipolytic reaction.

·· Screening of *Aspergillus flavus* and *Aspergillus parasiticus* strains for aflatoxins production according to Abramson and Clear (1996).

Aspergillus flavus and *A. parasiticus* isolated from camel milk were grown on potato dextrose agar media at 25°C for 5 days. Conidia suspensions of individual isolates were adjusted to approximately 10^5 conidia/ml. The suspension in 0.1 ml amount was used to inoculate 50 ml of yeast extract sucrose broth and incubated at 28°C for 14 days. The entire culture was blended and extracted with 50 ml of chloroform. The mycelial mat was separated from the broth by filtration through filter paper whatman number 12.5 cm. The mat was dried at 80°C over night and weighed to determine the mycelial mass. The filtrate was transferred to separating funnel and extracted using 50 ml chloroform. The same extraction

process was repeated three times. The extract was combined, evaporated to dryness and measured by using Enzyme Linked Immunosorbent Assay method (ELISA) for aflatoxin B1, B2, G1 and G2 production.

Results and Discussion

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

Camel milk 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 camel milk quality. The organoleptic properties of camel milk samples are shown in table 1. All the samples were generally opaque-white in color and with sweet pleasant taste. The flavor and odour, appearance and colour and bacteria were scored 43 ± 0.02, 18 ± 0.14 and 30 ± 0.10 points, respectively. The mean acidity % and pH value were 0.16 ± 0.01 and 6.70 ±0.1, the resazurine test was lilac colour. The 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, 2004).

Table 1. Organoleptic properties of camel milk samples.

Criteria	Score	± S.E.M.
Flavor and odor (45)	43	0.02
Color and appearance (20)	18	0.14
Bacteria (35)	30	0.10
Total%	91	0.05
Mean Acidity%	0.16	0.01
Mean pH value	6.70	0.1
Resazurine test	Lilac color	-

The results given in table 2 revealed that total *Psychrophilic, Mesophilic* and *Thermophilic* count were detected in 40%, 70% and 16% of the milk samples with a mean values $3.5x10^3$ ±$1.1x10^2$, $9.1x10^4$ ± $3.1x10^2$ and $4.9x10^3$ ± $2.3x10^2$ (cfu/ml) respectively. The samples contained higher *Mesophilic* count than *Psychrophilic* and *Thermophilic* count. Higher counts were recorded by Al-Mohizea (1986) and Sallam and Nagah (1993). This might be due to the physical environmental condition of desert, which allow 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 500000 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.

Results shown in table 3 demonstrate that *Aerobic spore former* bacteria were isolated from 30% of the samples with a mean value of $7.8x10^3$ ± $0.1x10^2$ cfu/ml. Higher counts were reported by Al-Mohizea (1986) and Sallam and Nagah (1993). *Aerobic spore former* bacteria are widely distributed in nature and may gain access to milk through various routes including air, water and utensils (John and Despencer, 2001). *Coliform* were present in 24% of the samples with a mean value of $9.5x10^4$ ±$2.6x10^3$cfu/ml. In general, *coliform* count may be used as indication of milk sanitation. The presence of more than 750 coliform/ml indicate bad hygienic measure during milk production, handling and distribution (Boor and Murphy, 2002). Moreover, about 30% of people in industrial countries and hundred of millions of people in developing countries suffer from diarrhoeal disease (Anonymous, 2000). Contamination of milk with coliform might induce many changes leading to economic losses (Robinson, 1990). The *Enterococcal* counts existed in 20% of the samples with a mean value of $3.2x10^3$ ± $0.04x10^2$. 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 gets heavily contaminated (Ray, 1996). *Pseudomonas* and *Aeromonas* count detected in 22% of the samples with a mean value of $3.2x10^3$ ± $1.5x10^2$ cfu/ml. *Pseudomonas* species are found predominantly in soil and water. They are commonly associated with milk spoilage through

Table 2. Total colony counts (cfu/ml) in raw camel milk samples (n=50).

Total colony	Total Samples	No. positive	%	Min	Max	Mean	±S.E.M.
PC	50	20	40	10	$7.3x10^4$	$3.5x10^3$	$1.1x10^2$
MC	50	35	70	10	$6.6x10^6$	$9.1x10^4$	$3.1x10^2$
TC	50	8	16	10	$2.5x10^4$	$4.9x10^3$	$2.3x10^2$

- PC: psychrophilic count
- MC: mesophilic count
- TC: thermophilic count

Table 3. Colony counts (cfu/ml) of different microorganisms in camel milk samples (n=50).

Tests	Total	No.	%	Min	Max	Mean	±S.E.M.
Aerobic spore *former*	50	15	30	10	$5.1x10^5$	$7.8x10^3$	$0.1x10^2$
Coliform count	50	12	24	40	$8.2x10^5$	$9.5x10^4$	$2.6x10^3$
Total Enterocoocai count	50	10	20	10	$7.1x10^4$	$3.2x10^3$	$0.04x10^2$
Pseudomonas *& Aeromonas*	50	11	22	10	$9.4x10^4$	$3.2x10^3$	$1.5 x10^2$
Stapylococcus count	50	9	14	10	$6x10^4$	$3.3x10^3$	$1.5 x10^2$
Total mold & yeast count	50	50	100	10	$4.7x10^7$	$6 x10^6$	$2.9 x10^3$

Table 4. Molds as well as their lipolytic activity found in 50 raw camel milk samples.

Isolated strains	No.	%	Lipolytic activity	
			positive	%
Genus : *Aspergillus*	**31**	**62**	**20**	**64.5**
A.flavus	**14**	**28**	**10**	**32.3**
A.parasiticus	**11**	**22**	**6**	**19.3**
A.sydowii	**5**	**10**	**4**	**12.9**
A. ustus	**1**	**2**	**0**	**0**
Genus : *Penicillium*	**18**	**36**	**16**	**88.9**
P. aurantiogriseum	9	18	8	44.4
P. funiculosum	5	10	4	22.2
P. purpurogenum	3	6	3	16.7
P. viridicatum	1	2	1	5.6
Genus : *Acremonium*	**16**	**32**	**9**	**56.3**
A.fusidiodes	10	20	5	31.3
A. strictum	6	12	4	25.0
Genus : *Alternaria*	**14**	**28**	**14**	**0**
A.alternata	9	18	9	0
A.tenuissima	5	10	5	0
Genus : *Chrysosporum*	**11**	**22**	**11**	**100**
C. carmichilli	7	14	7	63.6
C. keratinophilium	4	8	4	36.4

production of heat stable enzymes, protease and lipase inducing off-flavor and shortening shelf life time of milk (Celestino *et al*, 1996). Coagulase positive *Staphylococcus* was isolated from 14% of the samples with a mean value of $3.3x10^3 \pm 1.5x10^2$ cfu/ml. These results agreed to certain extent with those reported by Barbour *et al* (1985) and Sallam and Nagah (1993). It is potenial pathogens 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 nomades. 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 glucosaminidase (NAGase) were 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 milk samples.

Mold and yeast were isolated from all camel milk samples with a mean value of $6x10^6 \pm 2.9x10^4$ cfu/ml. *Aspergillus*, *Penicillium, Acremonium, Alternaria* and *Chrysosporum* were isolated samples at percentages of 62%, 36%, 32%, 28% and 22%, respectively. Ninety mold strains were tested for their lipolytic activity of which 70 strains could produce lipase enzyme which hydolysed the fatty compound tween 80 (poly exoethylene sorbitan monooleated) forming oleic acid precipitated as calcium oleate. Lipase production differed not only among the different mold species but also among the isolates in the same species (Table 4). Lower values were recorded by Sallam and Nagah (1993).

The analysis of mycoflora of samples showed that there were many saprophytic fungi inhibiting 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 also may constitute part of normal flora of camel wool (Bagy and Abdel-Hafz, 1985 and Laila *et al*, 1998). The presence of large number of molds in camel milk could constitute a public health hazard or may be responsible for undesirable changes and inferior quality of the produced 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; Hubbert *et al*, 1996 and Conesa *et al*, 2001). Lipase enzyme catalyses the hydrolysis of triglycerides of fatty acid yielding monoglycerides, 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, 2004). The high level of lipase enzyme may be responsible for temporal bitterness noted in some soft cheese manufactured from camel milk (Farah and Streiff, 1994; Abu-Tarboush *et al*, 1998; Giardet *et al*, 2000; Attia *et al*, 2001).

None of the *A. flavus* and *A. parasiticus* isolates from examined camel milk samples proved to be aflatoxin B1, B2, G1 and G2 producers.

A. flavus and *A. parasiticus* are pathogenic fungi which produce aflatoxins that render camel milk toxic to the consumer. Although they have world

wide distribution in nature, there are many factors that restrict their aflatoxin production in milk. The most important factors for aflatoxin production are moisture, relative humidity and temperature. The optimal growth of *A. flavus* and *A. parasiticus* occur at 37°C while the optimal aflatoxin production occur at 24-29°C (Abramson and Clear, 1996; Beucht *et al*, 1998; Chapman, 2003 and Jakobsen, 2004)). Therefore the camel milk is suitable for growth of fungi but not suitable for aflatoxin production.

Conclusion

The result of our study clearly showed the importance of camel milk examination, as revealed by potential problems associated with bacterial and milk quality. Although camel milk does not have pathogenic microorganisms but still harbour public health hazard to the consumer. Therefore, good sanitation and hygiene during milking and handling of camel milk are important factors to prevent milk spoilage and protect the consumers' health.

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