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Short Communication

Microbial quality of camel's raw milk in central & southern regions of United Arab Emirates

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Abstract: The goals of this study were to assess the possible hazards that might occur as a result of consuming camel's milk fresh, un-pasteurized. The study dealt with the microbial quality of camel milk, which includes detection of pathogenic bacteria, enumeration of microorganisms (bacteria-yeast-moulds) that may cause changes to the milk, the distribution of bacteria in camel milk. The microbial quality of camels raw milk was investigated, 50 samples were analyzed for: Aerobic plates count, total *coliform*, total *Staphylococcus aureus* total yeast and mold. Sixty eight samples were examined for *Bacillus cereus, Salmonella spp., Clostridium perfringens, and Listeria monocytogenus*. The results indicated that the mean value of aerobic plate count $1.8x10^5$cfu/ml, mean value of total coli form $6.8x\ 10^1$, mean value of *staphylococcus aureus* $1.2x10^3$, yeast mean value $4.1x10^{-1}$ cfu/ml. All samples tested for pathogenic were negative for *Salmonella spp., Clostridium perfringens, and Listeria monocytogenus, positive for Bacillus cereus, staphylococcus aureus, and Echerichea coli*. The distribution of bacteria in camel milk as follow: 43% for gram-positive cocci, 11% for gram-negative cocci, 30% for gram negative rods, 23% for gram positive rods, 32% for staphylococcus, 15% for yeast.

Key words: camel milk, microbial quality, U.A.E.

المحتوى الميكروبى لحليب الابل الخام فى الإقليم الأوسط والجنوبي لدولة الإمارات العربية المتحدة

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ملخص: هدفت الدراسة لتقيم الخطر الذى قد ينجم عن تناول حليب الابل الخام (أي الغير معامل حرارياً) وشملت دراسة المحتوى الميكروبى، والكشف عن البكتيريا المسببة للإمراض ومدى تواجدها فى الحليب الخام، كذلك توزيع البكتيريا في الحليب الخام و كانت النتائج كالاتى: المحتوى الميكروبى: العد الكلى الميكروبى 8 و1 $\times 10^5$ والعد الكلى لبكتيريا الكلييفورم 8 و6 $\times 10^1$، متوسط العد الكلى للبكتيريا العنقودية 2 و1$\times 10^3$ ، الخمائر 1 و4$\times 10$. البكتيريا المرضية: كانت النتيجة سالبة بالنسبة لبكتيريا السالمونيلا والكلوستيريديوم والليستيريا وموجبة للبكتيريا العنقودية والباسيلس والايشريشيا كولاي. توزيع البكتيريا فى الحليب الخام: كانت نسبة العزل الميكروبى من47 عينة كالاتى: 43 % كروية موجبة لصبغة جرام، 11% ، كروية سالبة لصبغة جرام، 30% عصوية سالبة لصبغة جرام ، 23% عصوية موجبة لصبغة جرهم، 32% بكتيريا عنقودية، 15% خمائر.

كلمات مفتاحية: حليب الابل ،المحتوى الميكروبى، دولة الامارات العربية المتحدة.

Introduction

Milk is an ideal habitat for the growth and multiplication of microorganisms due to its nutritional constitution which contain protein, carbohydrate, mineral and vitamins. All these components support the growth of many forms of bacteria. A raw milk aseptically drawn from a healthy animal usually contains a few bacteria (Frazler, 1967). Beside that milk is an ideal medium for the growth of microorganisms from surrounding environment (Cousins and Barmley, 1981). The microbial quality of camel's raw milk in Riyadh City was investigated by Al–Mohizea (1986) who found the aerobic plate count exceeded $_{10}^{5}$ cfu/ml in 13 samples and averaged $2.2x10^5$cfu/ml. All samples tested were found to contain coli form in excess of 10/ml with an average of $5.1x10^5$cfu/ml. Coagulate positive *Staphylococcus aureus* was detected in all samples with an average of $1.3x10^3$cfu/ml. *Salmonella, Bacillus cereus*, and *Yersinia enteroclitica* were sporadically detected in the same samples. Al-Saleh, et al. 1997 stated that the Pseudomonas flourescens which was isolated from camel raw milk (bulk milks) showed maximum growth and proteinase activity in *Trypticase soya* both (TSB) at 27°C after 32 and 24 h respectively. Optimum temperature was 37°C for growth, and 27°C, for proteinase production, however proteinase was produced at all temperatures studied (7–37°C). Proteinase production in (TSB) was enhanced by addition of skim milk or casein, but not by alpha–casein. The presence of amino acids or peptides was necessary for proteinase production. Growth and proteinase production were enhanced by aeration occurred over a wide pH range. Growth was maximum at pH 6.7–6.9 and proteinase production at pH 6.9. Addition of glucose at increasing concentrations up to 0.8%, enhanced growth, but had negligible effect on proteinase production, which was reduced by higher concentrations of glucose.

Farah, (1993) reviewed the ability of camel milk to inhibit growth of pathogenic bacteria and it's relations to whey lysozyme. Twenty of 200 samples collected from individual camels inhabited growth of one or more of six pathogenic test organisms. The milk samples with inhibition properties scored zero in the California mastitis test. The lysozyme content of the twenty samples showing growth inhibition was 648 µg/100ml which is significantly higher than the average in the 38 samples (62.8 µg/100ml) that has no inhibitory effect. Duhaiman (1988) purified camel milk lysozyme and separated it from lactoferrin and a low molecular weight protein. The Lytic effect of camel milk lysozyme was assayed using *Escherichia coli* and *Micrococcus lysodeikticus* and its activity was compared with that of lysozyme from human milk and egg white. The specific activity of camel milk lysozyme was found to be lower than that of lysozyme from the human milk or from egg white.

Camel milk has the ability to inhabit the growth of pathogenic micro-organisms because it contains number of enzymes with ant-bacterial and anti-viral properties these are: **Lactoferrin** which prevents microbial growth in the gut **Lacto peroxides** that suppresses gram-negative bacteria and most effective in raw milk during the first 4 days, **peptidoglycan recognition protein** (PGRP) that broad anti-microbial activity, stimulates the immune system, **N-acetyl-glucosaminidase** (NAGase) antiviral activity, **Lysozyme** which inhabits the growth of bacteria, and has effective influence on the storage camel milk, and **immunoglobulins** these possess several traits which give them tremendous advantage over conventional antibodies (Werney 2003). El-Agamy et al. (1992) extracted lysozyme, lactoferrin,

lactoperoxidase, immunoglobulin A, from camel milk. The activity of these protective proteins was assayed against *Lactococcus lactis sub-sp., Cremoruse, Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *rotavirus*. The antibacterial activity spectrum of camel milk was similar to that of egg white lysozyme and different from the lysozyme of bovine milk. Bovine and camel milk lactoferrin, antibacterial activity spectra were similar. Camel milk lacto peroxidases were bacteriostatic against the gram-negative cultures, the immunoglobulin had little effect against the bacteria, but high titers antibodies against rotavirus were found in camel milk.

The main objectives of this study were to (1) investigate the microbial quality of camel milk, (2) detect the pathogenic bacteria, (3) enumerate the bacteria that may cause changes in camel milk and the distribution of those bacteria.

Materials and Methods

This study was conducted in Al-Ain Food Laboratories, (Abu Dhabi Food Central Authority) , UAE. Camel milk samples were collected from 118 lactating camels, from two different private camel herds, one herd from Central region (Alrawi) and the other from the Southern region of (Al Ain) United Arab Emirates. All camels were in mid lactation (2^{nd} to 5^{th} month of lactation) 250 ml, sterilized plastic containers were used for the collection of samples.

The camels used for the milk collection were healthy and uninfected. The udder was cleaned and washed with a disinfectant solution (Safflon; 20% concentration). The samples were analyzed for total aerobic plate count, total coli form, total *Staphylococcus aureus*, and total yeast and mould. Also, samples were analyzed for the detection of *Bacillus cereus, Clostridium perfringens, Listeria monocytogenes*, and *Salmonella spp.* All the microbiological analysis was carried according to FAO (1992).

Aerobic plate count

For this test plate count agar (Himedia laboratories PVT. Limited) was used, plates were incubated for 48 hrs at 35 ^{o}C.

Enumeration of total coli forms

Presumptive test was done using MacConkey broth, and tubes were incubated at 37^{o}C, examined for gas production and growth after 24h. A confirmation test was done using BGB broth for total coliform, and L-EMB agar for E.coli and incubated at 35^{o}C for 18-24h.Two typical colonies from each L-EMB plate were picked and transferred to plate count agar slants for morphological and biochemical tests.

Enumeration of yeast and moulds

Dilution plating technique, with potato dextrose agar (Himedia laboratories PVT Limited) was used, plates were incubated at 22-25˚C for 5 days.

Enumeration of *Staphylococcus aureus*

Direct plate count method was used with Baird-Parker medium (Oxoid)

Detection of *B. Cereus*

Bacillus cereus agar (Oxoid CM 617) and *Polymaxin B* (50.000 units) was added to the *Bacillus cereus* selective supplementary then 50 ml of sterile Egg Yolk emulsion was added Incubated at 30°C for 2 hr. gram stains were made and APl 50 CHB system was used for identification. The confirmation test was done using *Bacillus cereus* agar and incubated at 30°C for 18-24 hrs. Gram stained smears were made and examined microscopically. *Bacillus cereus* will appear as large gram positive bacilli in short to long chains spores are ellipsoidal and central to sub-terminal. API strip was inoculated and incubated

at 30°C. The result was read according to the manufactures instructions.

Detection of *Clostridium perfringens*

Clostridium perfringens was detected according to the method of (ICMSF, 1988). Using Cooked Meat Medium (Oxoid.), Perfringens Agar (TSC&SFP) and Neomycm, 1% solution. Incubated for 2 4 hr at 35-37°C.

Detection of *Listeria monocytogenes*

Listeria monocytogenes was detected according to the method of FAO (1992). Using 1) Listeria enrichment broth base (Oxoid); 2) Oxford agar; plates were incubated at 35 ℃ for 24-8hr.

Detection of *Salmonella*

Salmonella was detected according to the method of FAO (1992). Pre enrichment medium (Buffer peptone water)-Enrichment broth (*Rapport-vassiliadis*) (RV)-Xylose *Lysine Descoxychalate* (XLD) agar (Himedia)-Triple sugar iron agar (TSI) were used, incubated at 35°C for 24hr. API 20E Test System was used for diagnostic

Isolation of Bacterial Cultures

The main groups of microorganisms and their components comprising the microflora of milk were detected by isolations, purification, and identification. The samples were cultured on different media e.g. Gram positive bacteria, cultured on Blood agar media, gram-negative bacteria cultured on MacConkey agar, *Staphylococcus aureus* on baird parker agar, *Bacillus cereus* on mannitol-egg-yolk-polymxin agar. *Escherichia coli* on Levine's eosin-methylene blue agar (L-EMB). Yeast cultured on Potato dextrose agar. The culture was inoculated by streaking. Inoculated media were incubated aerobically at different temperatures (25, 30, 35°C) for 24-48 hrs. The solid media plates were examined, microscopically for growth, colony morphology, and any change in the medium.

Purification of Bacterial Cultures

Pure cultures of bacteria were obtained by sub-culturing from a typical and well-isolated colony on solid media (isolation media) till pure bacterial growth was obtained.

Identification of bacterial cultures

For the identification of unknown bacteria the steps were followed:

1) Making of smears, stained with gram-staining technique according to Green berge et al. (1992). 2) Motility, motility of bacteria was studied by Hanging Drop Technique according to Cowan and Steel (1990).

Biochemical identification

API 20E: was used for identification of *Enterobacteriaceae*. - API 50 CHB for *Bacillus*. - ID 32 C for yeast. - ID 32 *Staph* for *Staph spp.* - ID 32 GN for gram-negative rods.

Results and Discussion

The aerobic plate count is an indication of the sanitary conditions under which the food was produced (Andrews, 1992).

Total Aerobic plate count (APC) values may range from >100ml^{1} to 1×10^{6} / ml of milk, consequently high initial APC values in milk e.g.>100,000 ml ^{1} are evidence of serious faults in production hygiene, where as the production of milk having APC values <20,000/ml reflects good hygiene practices (International Dairy Federation, 1974).

Table 1 shows the means the standard deviations and the ranges of bacterial counts of the camel's raw milk. The aerobic plate count varied from 5 x 10 ^{2} to 7.4 xl0^{5} cfu/ ml with an average of 1.8×10^{5} ± 2.3×10^{4} cfu/ml. Out of 50 samples tested, 22 samples were found to contain bacterial counts excess of 10^{3} cfu/ml. This was lower than that reported by Al-Mohizea (1986) for the

total aerobic count of camel's milk in Riyadh markets (2.2 x 10^5 cfu/ml), this may be due to that our samples were collected from individual farms, under highly controlled conditions. The Al-Mohizea samples were collected from markets, which might be from numerous farms. Table 1 also indicated the mean values, the range, and the standard deviation of total coliform in camel milk. It varies between 4 cfu/ml to 2.1x 102cfu/ml, with a mean value of 6.8 xl01 ±6.6x 10^1 .Out of 52 milk samples tested for coliform, 10 samples exceed 10^2 cfu/ml. This was considered low in comparison to that found by Al-Mohizea (1986) for Saudi Arabia camel milk (5.1x105 cfu/ml) and this generally provides an index of the sanitation used during collection. The presence of S aureus indicated contamination from the skin, mouth, or the nose of the food handler (FAO, 1992). The S. aureus count ranged from $2.1xl0^2$ to $7.2xl0^3$ cfu/ml with a mean value of $1.2x10^3$ cfu /ml (Table 1). All the samples tested were found negative for molds. Concerning the yeast 7 samples out of the (68) samples tested were found positive, and ranging from $1.3x10^2$ to $1.0xl0^2$ cfu/ml, with a mean value of $4.1x10^1$ cfu/ml (Table 1).

Table (2) shows the distribution of bacterial counts of camel's raw milk. Fifty samples were tested for total aerobic counts, 2 samples < 10^4 cfu/ml, 26 samples >10^4 cfu/ml, 22 samples >10^5 cfu/ml, Nil samples approached 10^6. Fifty two samples were tested for total Coliforms, 10 samples were <10 cfu/ml 32 samples were >10^1cfu/ml. And 10 samples were >10^2 cfu/ml. Twenty five samples showed positive for Staphylococcus aureus, 3 samples were <10^2 cfu/ml, 14 samples <10^3 cfu/ml, 8 samples <10^4 cfu/ml.

Table 3 shows results of the total aerobic count of dromedary camel milk with corresponding values for cow milk as reported by Mamoun (1981) for Sudanese cow milk. The value range from 5.4 x 10^5 to 6.7 x 10^5 cfu/ml. From the data on table 3 we can observe a wide variation between species. This wide variation may be ascribed to the failure in maintaining consistent sanitary conditions, and for storage temperature, (Cousin and Bramley, 1981).

Table 1. Mean counts (cfu/ml) of Aerobic Plate Count.

Microorganisms	Range	Mean	SD*
	CFU/ml		
Total Aerobic	$5 \times 10^2 – 7.4 \times 10^5$	1.8×10^5	2.3×10^4
Total coliform	4cfu/ml _ 2×10^2	6.8×10^1	6.6×10^1
Total Staph.	$2.1\times10^2 – 7.2\times10^3$	1.2×10^3	1.5×10^2
Total yeast	$1.3\times10^1 – 1.0\times10^2$	4.1×10^1	3.1×10^1

* SD =Standard deviations

Table 2. Distribution of bacterial count of camel's raw milk.

	Aerobic plate count (cfu/ml)				Total *coliform* (cfu/ml)			Total *aureus* (cfu/ml)		
	<10^3	>10^4	>10^5	>10^6	<10	<10^2	<0.3x10^4	<10^2	<10^3	<10^4
No. of samples	2	26	22	Nil	10	32	10	3	14	8
%	4	52	44	0	19.2	61	19.2	12	56	32

Table 3. Total bacterial count of camel milk compared with cow milk (Range and mean values).

	Present study	Camel*	Cow [a]
Ranges	$5.\times10^2$ - 7.4×10^5	1.7×10^2 - 5×10^6	5.4×10^5 - 6.7×10^5
Means	1.8×10^5	2.2×10^5	-

* Adapted from Al-mohizea (1986)
a Adapted from Mamoun (1981)

Pathogenic Bacteria in raw camel milk

Raw camel milk may contain microorganisms pathogenic for man, and their source may lie either within or out side the udder. Pathogenic bacteria may present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis are *Staphylococcus aureus*, and *Escherichia coli*, and all are pathogenic Sinell (1973).Contamination of raw milk by pathogenic bacteria from source external to the udder may be caused by *salmonella*e strains, which produce many out breaks of *enteritis* Robinson et al. 1979.

Table 4 shows some pathogenic bacteria isolated from camel raw milk. Sixty eight samples were tested, for the detection of *Staphylococcus aureus*, *Bacillus cereus,* and *Listeria monocytogens*, and *Salmonella spp., Clostridium perfringens.* All the samples tested were negative for *Listeria monocytogens, Salmonella spp.*, and *Clostridium perfringens.* Twenty one percent of the samples were found positive for *Bacillus cereus.* All the samples tested were found positive for *Staph. Spp*. and 37% of these samples were found positive for *Staphylococcus, aureus*. Our findings were comparable to that found by Al-Mohizea (1986), the only difference is that he found *S. aureus* in all samples he tested. The percent in this study is low compared to the percentage of *S. aureus* found in bovine milk (42%) as reported by Elnazier (2000) for raw bovine milk at Soba in Khartoum States, which ranged between Nil to > 2000 cfu/ml. These negative results against the occurrence of most pathogenic bacteria, might be due to the activity of protective proteins (Lysozyme, lactoferrin, lactoperoxldase, immunoglobulm G and A) of camel's milk, as reported by Barbour et al. (1984), and El-Agamy, (1992), who found that camel milk lysozyme (LZ) was effective against *Salmonella*, but ineffective toward Staph aureus, and that camel milk *Lactoperoxidase* was *bacteriostatic* against the gram-positive strains, and was bactericidal against gram-negative cultures.

The total aerobic bacterial content of raw camel milk

Many types of bacteria was isolated and identified, with varying isolation levels from 46 samples. The bacterium that was isolated was:

The gram-positive cocci

Staphylococci: were isolated from 15 milk samples. The average rate of isolation from all samples was 31.91% (Table5). The named species are *Staphylococcus aureus* (Ref. 8), *Staphylococcus capre* (Ref. 7), *Staphylococcus hyicus* (Ref. 22), and *Staphylococcus xylosue* (ref 6) (the most dominant one)

Other gram-positive cocci

e.g. *Streptococcus, Micrococcus,* Aerococcus were isolated from 20 milk samples. The average rate of isolation from all samples was 42.55% (Table 5).

The named species is *Aerococcus viridians* (Ref. 43).

The gram-positive rods

Bacillus species: were isolated from 11 milk samples. The average of isolation from all samples was 23.4%.

The gram-negative cocci were isolated from 5 samples. The average rate of isolation from all samples was 10.6%.

The gram-negative rods were isolated from 14 milk samples. The average rate of isolation from all samples was 29.8%. The named species are *Aeromonus salm salmomcida*(Ref. MA10), *Escherichia coli*, *Enterobacter* cloacae, and *Eenterobacter aminigenus*. Ahmed (1995) isolated the same types of bacteria from cow milk and he found that the average rate of isolation was 51.1% *Staphylococcus*, 29.6% *Streptococcus*, 6.6% *Micrococcus*, 13.16% gram-positive rods, and 2.27% gram-negative rods.

Yeast was isolated from 7 milk samples. The rate of isolation from all samples was 14.9%. The named species are *Candida ciferri* and *Candida guilliermondii* (Ref. 9).

Table 4. Frequency Occurrence of some pathogenic bacteria in raw camel milk.

Pathogenic bacteria	No. of sample tested	No. of positive samples	%
Staph. aureus	68	25	36.8
Bacillus cereus	68	14	20.6
Listeria monocytogen	68	-	0
Salmonella spp.	68	-	0
Clostridium perfringens	68	_	0

Table 5. Types of bacteria isolated from raw camel milk.

No. of samples	Organisms	+ve samples	%
47	Gram+ ve cocci	20	42.55
47	Gram + ve rods	11	23.40
47	Gram - ve cocci	5	10.64
47	Gram-ve rods	14	29.79
47	Staph. Spp.	15	31.91
47	Yeast	7	14.89

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