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MICROBIOLOGICAL CHARACTERISATION OF ONE HUMPED CAMEL MILK IN MOROCCO

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ABSTRACT

In the present study fresh camel milk (*Camelus dromadarius*) were collected from camel farms in the region of Al-Kalaa Sraghna (Morocco). All samples were transported to the laboratory at 4°C and analysed on the same day for their microbiological characteristics which included: standard plate count (SPC), total and faecal coliforms, enterococci, staphylococci, lactic acid bacteria and yeasts. Results showed that the microbial profiles were relatively low for all the microorganisms studied. The average SPC was 5×10^4 cfu/ml, staphylococci numbers ranged from less than 1 cfu/ml to 5×10^3 cfu/ml. Enterococci reached an average of 20 cfu/ml. Coliforms were the most abundant microorganisms in camel milk and ranged from less than 1 cfu/ml to 8×10^4 cfu/ml. 33.33 % of staphylococci isolated were coagulase positive and among the isolates collected from all samples no *E.coli* was detected. Lactic acid bacteria counts in the samples showed an average of 10^4 cfu/ml while yeasts ranged from less than 1 cfu/ml to 9×10^4 cfu/ml.

Key words: Camel milk, hygiene, microbiology, quality, safety

The camel population of Morocco numbers about 84,845 (MAMVA, 1993). Camels are domesticated in Morocco mainly for their meat and milk. Camel milk is gaining more popularity, as an important component of human diet throughout the world. It may contain all essential nutrients found in milk from other species (Farah, 1993).

Most camel milk is consumed as raw or after mild souring. The contaminating hazardous microorganisms including toxigenic species influence the safety of raw milk. The microbial load is among the various factors influencing the quality of milk.

The most important hazardous bacteria are *Salmonella*, *E. coli*, *L. monocytogenes*, *Campylo-bacter jejuni*, *Yersinia enterolitica* and *Staphylococcus aureus* (Adesiyun *et al*, 1995; Hahn, 1996; Graat *et al*, 1997 and Heeschen, 1997). All these species are of a considerable public health concern especially with foodstuffs consumed without any treatment (pasteurisation or sterilisation).

Data on camel milk is mostly limited to the broad chemical composition (Farah, 1993) and very few studies focused on the bacteriological quality of camel milk (Al-Mohizea, 1986; Teshager and Bayleyegn, 2001). The purpose of the present study is to evaluate the bacteriological quality of raw camel milk in Morocco.

Materials and Methods

Samples collection **:** A total of 17 milk samples were collected from the camels located in the region of Al-Kalaa (Morocco). Fresh milk samples were placed in an icebox and transported to the laboratory for the microbiological analyses, which were done immediately.

Standard Plate Count (SPC) : Appropriate serial dilutions (10^{-1} to 10^{-6}) of the samples in saline water (8.5% NaCl) were pour plated on standard plate count agar (PCA) (Biokarr, France). The plates were incubated at 37°C for 48 hours.

Coliform counts : Coliforms were enumerated on deoxycholate agar (Merck, Germany). The plates were incubated at 37°C for total coliforms and at 44°C for faecal coliforms for 24 hours. Isolated colonies were cultured on trypticase soya agar slants and incubated for 24 hours for further identification. Cultures were stored at 4°C until identification.

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Staphylococci

Dilutions up to 10^{-6} were plated on mannitol salt agar (Merk, Germany). The plates were incubated at 37°C for 24 hours. The small yellow colonies on the medium were counted and checked for their catalase and gram reactions. Catalase positive and gram positive colonies were spread cultured on trypticase soya agar slants for further characterisation.

Enterococci

The MPN (most probable number) using 3 tubes per dilution (10^{-2} to 10^{-4}) was determined on Azide Dextrose Broth (Difco Laboratory, USA). Incubation was done at 37°C for 24 hours. Tubes that had shown growth were propagated on Ethyl Violet Azide broth (Difco Laboratory, USA) and incubated at 37°C for 24 hours. Positive tubes were revealed by growth and formation of a violet precipitation in the bottom of the tubes. The number of positive tubes is reported to the table 1 for the most probable number of enterococci in the sample.

Salmonella

25 ml of the sample were added to 100 ml of sterile buffered peptone water (BPW) and incubated for 18 hours at 37°C. Two tubes of tetrathionate broth and 2 tubes of selenite cystein broth (Merck, Germany) were inoculated with 1 ml from the BPW and incubated for 24 hours at 37°C. Positive tubes of both media were streaked on Hektoen agar (Merck, Germany). The method described by Poelma *et al* (1984) was used for the identification of the suspected colonies blue green white with or without dark centre.

Spore forming bacteria

The initial dilution was heat activated at 80°C for 10 min and immediately cooled in ice water. Anaerobic sulfite reducing *Clostridium* were grown on SPS medium (Merck, Germany) in tubes which were then inoculated with 2, 1 and 0.5 ml of the heat activated dilution and incubated at 30°C for 24 hours. Dark colonies were counted.

Results and Discussion

Standard plate counts of fresh camel milk samples ranged from 10^4 to 2×10^5 cfu/ml with an average of 5×10^4 cfu/ml. The poor sanitary conditions indicate that the level of SPC could be higher than the values found. These profiles were approximately similar to those observed by Teshager and Bayleyegn (2001) in Ethiopia, but were higher than those observed by Al-Mohizea (1986) in Saudi Arabia who reported an average of 2.3×10^3 cfu/ml.

Faecal coliform counts ranged from less than 1 cfu/ml to 10^2 cfu/ml, while total coliform profiles showed an average of 3×10^3 cfu / ml. Coliforms in our samples presented a relatively higher profiles than those reported by Teshager and Bayleyegn (2001) (6.2×10^3 cfu/ml) and Al-mohizea (1986) (2.09×10^2 cfu/ml).

Enterococci were unexpectedly found in very low numbers in most samples with an average of 20 cfu/ml. Staphylococci counts reached an average of 10^3 cfu/ml in fresh milk samples. These averages are still insufficient (not high enough) to induce a risk of intoxication by staphylococci toxin production (Bergdoll, 1970) and are still lower than the limits accepted in USA and EEC for the raw cow milk.

The determination of microorganisms involved in milk biochemical process included lactic acid bacteria and yeasts. Lactic acid bacteria counts ranged from 2×10^2 cfu /ml to 2×10^4 with an average of 10^4 cfu/ml, whereas yeasts reached the same average counts. As it is reported by Desmasures (1997) cow milk may have roughly the same counts for yeasts (3×10^2) and lactic acid bacteria. But in the case of camel milk, lactic acid bacteria cannot grow and produce sufficient acid, which may lead to curdling, as it is the case in cow milk. This phenomenon leads some searchers to use enzymes for the coagulation of camel milk.

The presence of lactic acid bacteria in high numbers are undesirable in fresh cow milk

Table 1. IMViC tests for the identification of coliforms strains isolated from camel milk.

Tests	Sample									
	1	**2**	**3**	**4**	**5**	**6**	**10**	**13**	**14**	**16**
I	-	-	-	-	-	-	-	-	-	-
MR	+	-	+	-	-	-	-	-	+	-
VP	-	+	-	+	+	+	+	+	-	+
C	+	+	+	+	+	+	+	+	+	+
Genre	Cit	Ent	Cit	Kleb	Kleb	Ent	Kleb	Kleb	Cit	Ent

I : Indole Production ; MR : Methyl Red Test; VP : Voges Proskauer Test; C : Citrate Utilisation; Cit : *Citobacter* spp, Ent : *Enterobacter* spp, Kleb : *Klebsiella* spp.

Table 2. Biochemical identification of *Staphylococci* strains isolated from camel milk.

Tests	Sample													
	1	2	3	4	5	6	7	8	9	10	13	14	15	16
Gram colouration	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coagulase	+	+	+	-	-	-	-	-	-	-	-	-	-	
DNAase	+	+	+	-	-	-	-	-	-	-	-	-	-	
Thermonuclease	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Species	*S. aureus*	*S. aureus*	*S. aureus*	S. spp	S. spp	S. spp	S. spp	S. spp	S. spp	S. spp	S. spp	S. spp	S. spp	S. spp

S. aureus: Staphylococcus aureus, S.spp *: Staphylococcus* spp

because of the acid production and coagulation which is a default in fresh milk. About 33.33% of *staphylococci* isolated from Mannitol Salt Agar were coagulase positive. Similar results were reported by Teshager and Bayleyegn (2001).

The high counts of coliforms and high SPC may be explained by the contamination of camel milk during milking (by hands, utensils, udder, etc). Because of the low production collection and pasteurisation of camel milk is not easy to apply.

Microbiological determinations in the different samples indicated an acceptable bacteriological quality, Comparative to other milk species (especially cow) with regard to hazardous microorganisms profiles.

The low profiles of hazardous micro-organisms in camel milk might be due to the presence of some antibacterial proteins (lysozymes, lactoperoxidases, lactoferrin and immunoglobins) (Reiter, 1985). Some factors involved in microbial growth delaying such as lysozymes are present in high concentrations in camel milk compared to cow milk. The former may have 288 µg/100 mL, whereas the later has only 13 µg/100 mL (Vakil *et al*, 1969).

Al-Agamy *et al* (1992) showed that lysozyme and lactoferrin and lactoperixidase extracted from camel milk have an antimicrobial activity against *E. coli*, *S. aureus* and *Salmonella*. The higher activity of these inhibitory factors in camel milk showed that camel milk had a higher lysis activity than bovine milk on *Micrococcus lysodeikticus* (Al-Agamy *et al*, 1996) and *Escherichia coli* (Duhaiman, 1988).

Lactoferricin, a peptide cleaved from lactoferrin by pepsin, also presents an antimicrobial activity that originate from a direct interaction with bacterial surface (Tomita *et al*, 1991). Lactoferricin showed a marked growth-inhibiting effect on several bacterial strains including *E. coli*, *Klebsiella pneumonia*, *Salmonella enteritidis*, *Staphylococcus haemolyticus*, *Streptococcus thermophilus*, *Corynebacterium ammoniagenes*, *Bacillus subtilis* or *Bifidobacterium infantis* (Tomita *et al*, 1994).

From a technological point of view, the antimicrobial activity of camel milk can be exploited to improve preservation conditions of milk. But in the same time antimicrobial properties would make its technological adaptation to transformation into cheese and other fermented dairy products, more complex because of its low aptitude to the acidification.

Camel milk's antimicrobial properties can also explain the traditional therapeutic uses of this milk in folk medicine in different areas in the world (Yagil, 1982).

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