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Evaluation of physicochemical properties and microbiological quality of camel milk from Egypt

Abstract

So far, there has been little available literature regarding the quality and safety of consumed raw camel milk. Therefore, the present study was delineated to assess the physico-chemical properties and the bacterial contaminants of camel milk being collected from different geographic locations in Egypt. A total of 150 of raw camel milk samples were purchased from three different Egyptian Governorates during the period between September 2015 to August 2016. The collected samples were used for evaluating physicochemical properties, microbiological analysis. In general, a great variation in the chemical composition was found in the present study. Out of the tested milk samples, 133 were found to be contaminated with the total bacterial count, 69 samples were found to be contaminated with total *coliform* with a mean count of $3.70x10^4 \pm 1.20x10^4$, 51% of the examined samples were found to be contaminated with *Enterobacteriaceae* with mean count of $2.91x10^4 \pm 6.20x10^3$cfu/ml, 57 samples were found to be contaminated with *staphylococcus spp*. while *staphylococcus aureus* was identified in only 22 samples with an average of $7.30x10^2 \pm 2.60x10^2$. The results herein indicated that camel milk had inferior microbiological quality due to its high contents of total bacterial counts, total *coliform*, total *Enterobacteriaceae* and *staphylococcus spp*. which are not in conformity with the official standards. These pathogenic germs can limit the keeping quality and safety of raw camel milk. There is a pressing need to improve the hygienic condition and providing adequate sanitary measures from the stage of production till milk consumption.

Keywords: camel milk, bacterial counts, chemical composition, sanitary assessment, public health hazards

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Introduction

Camelus dromedarius, one-humped camel, plays a significant socio-economic role in dry and semi dry zones of Asia and Africa.[1] For thousands of years, camels have been integrated into the daily life of nomads and reared under harsh conditions. Currently, camel remains a highly valued animal for its meat, milk, wool, skin, and folk medicine. It also serves as a mean of transportation, sport as well as a source of pride and wealth.[2] The population of the Arabian one-humped camel is approximately 25 million, of which, 159 thousands raised in Egypt.[3] Camel milk is considered as an integral source of food in Egypt particularly for nomadic people who live in arid regions such as Kirdasa, Ismailia, Matrouh and Shalateen.[4] The value of camel milk has recently received a particular attention worldwide due to its high therapeutic value for human health.[2] Camel milk is unrivaled from other ruminant milk in terms of composition as it contains high concentrations of immunoglobulin's and insulin, high levels of essential elements such as sodium, potassium, iron, copper, zinc and magnesium and vitamins especially thiamine (B1), riboflavin (B2) and ascorbic acid (C) but low in protein, sugar and cholesterol.[5] Various therapeutic properties have been reported for camel milk including anti-hypertensive, antioxidant, antimicrobial, immunomodulatory and anti-thrombotic.[6–8] It has also been therapeutically used to combat various diseases such as dropsy, jaundice, tuberculosis, asthma and leishmaniasis.[3,5,9] Pastoralists often consume this milk in a raw state either fresh or in varying degrees of sourness thereby representing a major concern for public health and is likely cause food-borne diseases.[2–8] Although camel milk has received a growing interest during recent years, there has been paucity information regarding its quality and the potential bacterial contamination. Therefore, the purposes of this study were to assess the sanitary, physico-chemical composition of camel milk being collected from three different Egyptian Governorates as well as to evaluate its bacterial contaminants.

Materials and methods

Sampling

A total of 150 samples of raw camel milk were purchased from three different Egyptian Governorates (Giza, Matrouh and Shalateen) during the period from September 2015 to August 2016. The milk samples were aseptically taken in its commercial packages, labeled, and placed in cool box then transported to the laboratory of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University. The collected samples were divided aseptically into three portions to be used for sanitary analysis, chemical analysis and for microbiological analysis.

Physico-chemical properties of camel milk

The titratable acidity of the collected milk was determined according to the standard methods described by[11] Official methods of analysis. The pH, dry matter, fat content, ash, total solids (T.S), solids not fat (S.N.F), lactose and protein of milk samples were measured using Lacto scan (MCCW Milk Analyzer, 8900 Nova Zagora, Bulgaria using). The percentage of moisture was also calculated by subtracting total solids (T.S) % from 100.[11]

Microbiological analysis

Milk samples were subjected to microbiological examination to

determine the potential bacterial load, total bacterial count (TBC), total *coliform* count (CC), total *Enterobacteriaceae* count, total *staphylococcus* count and *staphylococcus aureus* count.

Bacterial enumeration

Initially, 25ml of each raw milk sample were dispensed in a sterile flask that contains 225ml 0.1 % peptone water and mixed thoroughly. A subsequent serial decimal dilution of each sample was prepared in 0.1 % peptone water[12] International Organization for Standardization.

Total bacterial count

The total bacterial count was performed according to the standard procedures described by.[13] The procedure was done in duplicate using standard plate count agar (Oxoid, UK). Colonies were counted after incubation at 37°C for 24-48 hours.

Total *Coliform* and *Enterobacteriaceae* count

Total *coliform* and total *Enterobacteriaceae* count were carried out using Violet Red Bile Agar and Violet Red Bile Glucose Agar, respectively according to the previously described methods[13] and the plates were incubated for 24 hours at 37°C. Pink colonies surrounded by bile precipitation were counted as *coliform*s, while pink to red purple colonies surrounded by red zone of precipitated bile was considered as *Enterobacteriaceae*.

Enumeration of *Staphylococcus spp.* and *Staphylococcus aureus*

Cultivation of *Staphylococcus spp*. and *Staphylococcus aureus* count were carried out on Baird Parker agar supplemented with egg yolk tellurite emulsion using the direct plate count method.[14] For Staphylococcus aureus, black shiny colonies surrounded by hollow zone were counted and confirmed by the coagulase test[15] (International Dairy Federation) and the presence of thermo nuclease activity on Toluidine blue O-DNA agar (Sigma, St Louis, MO, USA)[15] (International Dairy Federation).

Statistical analysis

The results for bacterial enumeration were logarithmically transformed into log10 for statistical analysis. The statistical analysis was done using ANOVA and Chi-square. The different microbial variables were performed using SPSS software (Version 10, SPSS Inc., Chicago, USA).

Results and discussion

Camel milk has been acknowledged for long time as a white gold of the desert because of its valuable nutritional and medicinal properties. In this study, we aimed at evaluating the physicochemical characters as well as the microbial contaminants of camel milk being collected from different areas in Egypt. Here, the physical properties of the examined milk samples were presented in Table 1. In short, the values of milk pH were ranged between 4.96-7.06 with the highest grand mean value of pH was 6.61 with standard error of 0.04 in the milk purchased from Giza. According to Food Agriculture Organization, the value of pH in fresh camel milk is ranged between 6.5-6.7.[16] The pH value recorded in the present study was consistent with those reported in Egypt by several authors[17–20] with respective values of 6.65, 6.64, 6.6, and 6.6. In contrast, other researchers have reported lower values[21–23] (5.43, 5.97, and 5.87), respectively. Similar pH values were also reported in other studies from different countries.[24–26] It has been reported that the pH value could play a significant role in determining the product quality. The titratable acidity of camel milk is the measure of lactic acid formed in camel milk. In the present study, the acidity among the examined samples was varied from 0.09-0.30% W/V, the highest grand mean value of the titrateable acidity was 0.18 % with standard error of 0.006 being determined in the milk that purchased from Shalateen area. Some of the examined samples, in particular, those from Shalateen express relatively a low pH value with high titratable acidity being attributed to the production of lactic acid by microbial flora. That was more likely during the summer season where ambient temperatures were relatively high and the lack of refrigeration for several hours during milk transportation.

Table 1 Values of some physical parameters in the tested raw camel milk collected from some Governorates in Egypt

Source	pH				Titratable acidity			
	Min. value	Max. value	Mean	SE	Min. value	Max. value	Mean	SE
Giza (n=50)	5.83	7	6.61	0.04	0.1	0.27	0.15	0.006
Matrouh (n=50)	6	7.06	6.59	0.039	0.09	0.21	0.16	0.005
Shalateen (n=50)	4.96	6.99	6.36	0.067	0.1	0.3	0.18	0.006
Total (n=150)	4.96	7.06	6.52	0.03	0.09	0.3	0.16	0.004

Composition analysis of camel milk

The results of chemical composition as well as the averages values of the examined samples were summarized in Tables 2, Table 3. Briefly, the percentages of chemical variables were ranged as follow: T.S (5.90–to 20.51), proteins (1.10–9.87), fat (1.36–7.00), lactose (2.27–7.15), ash (0.35–1.24) and moisture (79.00–94.10). The chemical composition of camel milk samples from previous literature in Egypt were given in Table 4. In general, a great variation in the chemical composition was found in the present study. It becomes clear that the overall milk composition can be influenced by several factors including physiological stage, feeding strategy, feed and water quality and quantity, seasonal variations, genetic, breed variation (within a species, herd to herd), stage of lactation and the health status. This view was in line with that given in several studies.[2,16,27,28]

Total bacterial content (TBC) of camel milk

The presence of various microbial groups in the examined raw camel milk was summarized in Table 5–7. In short, the total bacterial count was varied from 1.91 $x10^4$ to 4.68$x10^8$cfu/ml with an average of 1.82 $x10^7$ and standard deviation of 3.87$x10^6$cfu/ml. Generally most

tested samples (133/150) were found to be contaminated with the total bacterial which exceeds 10^6cfu/ml. In that context, nearly similar results were previously reported by several authors elsewhere.[29–34] In contrast, a lower bacterial burden was given in other studies.[20,35,36] In fact, The TBC of camel milk has been reported with values varied from 10^2 to 10^8cfu/ml.[29,37–39] The high TCB of the tested milk samples could be likely attributed to improper handling of the samples during collection, transportation, or even during storage.

Table 2 Chemical composition of the tested raw camel milk collected from some Governorates in Egypt

Source	Fat Mean±SE	Protein Mean±SE	Lactose Mean±SE	T.S Mean±SE	S.N.F Mean±SE	Ash Mean±SE	Moisture Mean±SE
Giza (n=50)	3.75±0.15[a]	2.61±0.11[b]	4.59±0.15[a]	11.65±0.36[a]	7.90±0.23[b]	0.70±0.01[ab]	88.6±0.71[b]
Matrouh (n=50)	3.79±0.17[a]	3.11±0.08[a]	4.81±0.15[a]	12.40±0.37[a]	8.60±0.23[a]	0.69±0.01b	87.74±0.34[b]
Shalateen (n=50)	2.57±0.15[b]	2.34±0.20[b]	4.69±0.12[a]	10.31±0.36[b]	7.74±0.22[b]	0.73±0.02a	89.69±0.36[a]
P value	0.0001	0.0004	0.5399	0.0003	0.0208	0.0863	0.0079

[a, b] Variables with different superscript within the same column are significantly different at $P \leq 0.05$

Table 3 Statistical analysis results of chemical examination of examined samples from Egypt (n=150)

Chemical composition	Minimum	Maximum	Mean±SE
Fat	1.36	7	3.40±0.10
Protein	1.1	9.87	2.70±0.08
Lactose	2.27	7.15	4.70±0.08
Total solids	5.9	20.51	11.50±0.22
Solid not fat	4.13	14.95	8.10±0.14
Ash	0.35	1.24	0.70±0.01
Moisture	79	94.1	88.60±0.21

Table 4 Exhaustive references (n =18) on camel milk composition from the literature in Egypt

Fat	Total protein	Lactose	Dry matter	Ash	Reference
3.8	3.5	3.9	12	0.8	[49]
3	3.9	5.5	13.2	0.8	[47]
5.5	4.5	3.4	14.4	0.9	[48]
2.9	3.7	5.8	13.1	0.7	[49]
3.6	3.27	5.53	13.2	0.8	[33]
3.6	3.05	4.4	11.95	0.9	[50]
3.9	3.1	4.47	12.36	0.8	[51]
3.95	3.26	4.74	12.8	0.85	[52]
4.2	3.27	4.31	12.95	0.75	[53]
3.78	3.3	5.85	15.06	0.7	[54]
3.55	3.01	3.48	13.75	0.93	[55]
3.33	3	3.33	12.75	0.81	[21]
4.4	2.91	3.18	11.3	0.9	[56]
4	3.46	4.86	13.2	0.87	[18]
5.6	3.55	4.24	14.13	0.87	[57]
3.49	4.07	5.94	15.93	0.91	[58]
4	4.4	5.1	14.3	1.01	[20]
3.3	3.16	4.94	12.21	0.87	[37]

Total *coliform* and *Enterobacteriaceae* count

In the present study, a total of 69 out of 150 (46%) of examined milk samples were found to be contaminated with total *coliform*, the maximum count was $1.65x10^6$ with a mean count value of $3.70x10^4 \pm 1.20x10^4$ (Table 5–7). Our findings were nearly similar to those given by El-Ziney MG et al.[40] However, several authors have reported lower levels of contamination rate.[20,35,41,42] On the contrary, a high mean count was determined by others.[29,30,33,34,37] On the other side Bassuony IN et al.,[32] reported a much higher detection rate of *coliform*s (85.7%) out of 35 raw camel milk samples from Matrouh Governorate, Egypt. Our findings demonstrated that 51% of the examined samples were found to be contaminated with *Enterobacteriaceae* with mean count of $2.91x10^4 \pm 6.20x10^3$cfu/ml with a maximum count of $6.70x10^5$cfu/ml. In the same context, comparatively various counts were recorded in several studies.[30,32,40,42] The *Enterobacteriaceae* and *coliform* bacteria being identified in the tested milk samples are considered as indicators of a potential fecal contamination. It has been reported that the existence of high numbers of these bacteria is commonly used as an indicator of poor hygiene, improper handling.[32] It is also remarkable to underline that food poisonings cases may happen when the numbers of theses bacteria is increased.[43]

In the present study, 57 out of 150 raw milk samples were found to be contaminated with *staphylococcus spp*. with maximum count of $3.20\ x10^5$cfu/ml and mean value of $7.70x10^3 \pm 2.60x10^3$. Importantly, *staphylococcus aureus* was identified in only 22 samples out of 57 staphylococci with an average of $7.30x10^2 \pm 2.60x10^2$ while the maximum count was $2.20x10^4$cfu/ml. Coagulate positive (CPS) and coagulase negative staphylococci were recovered in a percentage of 15 and 38, respectively and might be main reason for subclinical mastitis in dromedaries. Occurrence of staphylococcus sp. were previously reported by several authors.[30,44,45] But higher average counts of *staphylococcus aureus* were previously identified by others.[32,34] The presence of *S. aureus* in the examined raw milk samples could represent a potential health hazard. It has also been suggested that this bacteria represent the third most important cause of disease in the world among the reported food borne illnesses due to its capability to produce a wide range of heat stable enterotoxins.[46] In general *S. aureus* can gain access to milk either by direct excretion from infected udders (clinical or subclinical staphylococcal mastitis) or by contamination from the environment during handling and processing of raw milk.[46] Taken altogether, our results might indicate a poor sanitary condition under which the camel's milk was produced,

as well as an environmental contamination through several sources including contamination of the camel udder, mixing of evening and morning milk, pooling of milk from different suppliers and exposure during marketing. Hence, the consumption of raw camel milk could be a potential public health concern and is likely to cause food-borne diseases and the natural antimicrobial factors can only provide a limited protection against specific pathogens.

Table 5 Comparisons of bacterial load of examined raw camel milk samples collected from some Governorates in Egypt

	Giza n=50					Matrouh (n=50)					Shalateen (n=50)				
	pos	%	Min	Max	Mean±SE	pos	%	Min	Max	Mean±SE	pos	%	Min	Max	Mean±SE
Total bacterial count	50	100	$1.91x10^4$	$6.82x10^7$	$1.22x10^7 \pm 2.40x10^6$	50	100	$1.56 x10^5$	$6.82 x10^7$	$1.60x10^7 \pm 2.50x10^6$	50	100	$1.50x10^6$	$4.68x10^8$	$2.60x10^7 \pm 21.10x10^7$
Total *coliform* count	22	44	ND	$3.03x10^5$	$2.94x10^4 \pm 9.89x10^3$	26	52	ND	$6.20x10^5$	$2.70x10^4 \pm 1.32x10^4$	21	42	ND	$1.65x10^6$	$5.40x10^4 \pm 3.40x10^4$
Total *Enterobacteriacae* count	24	48	ND	$2.24x10^5$	$2.70x10^4 \pm 7.73x10^3$	29	58	ND	$6.70 x10^5$	$2.99x10^4 \pm 1.40x10^4$	24	48	ND	$3.40x10^5$	$3.03x10^4 \pm 9.80x10^3$
Staphylococcus spp. count	17	34	ND	$3.20x10^5$	$1.16x10^4 \pm 6.73x10^3$	18	36	ND	$6.00x10^4$	$2.50x10^3 \pm 1.20x10^3$	21	42	ND	$9.00x10^4$	$8.60x10^3 \pm 12.80x10^3$
Staph.Aureus count	6	12	ND	$1.70x10^4$	$6.12x10^2 \pm 4.11x10^2$	5	10	ND	$1.30x10^4$	$3.12x10^2 \pm 2.60x10^2$	11	22	ND	$2.20x10^4$	$1.30x10^3 \pm 5.90x10^2$

Table 6 Statistical analysis results of bacteriological examination of raw camel milk samples (n=150) collected from some Governorates in Egypt

Microbial counts	positive		Minimum	Maximum	Mean±SE
	No	%			
Total bacterial count	150	100	$1.91 x10^4$	$4.68x10^8$	$1.82 x10^7 \pm 3.87x10^6$
Total *coliform* count	69	46	ND	$1.65x10^6$	$3.70x10^4 \pm 1.20x10^4$
Total *Enterobacteriacae* count	77	51	ND	$6.70x10^5$	$2.91x10^4 \pm 6.20x10^3$
Staphylococcus count	57	38	ND	$3.20x10^5$	$7.70x10^3 \pm 2.60x10^3$
Staph. aureus count	22	15	ND	$2.20x10^4$	$7.30x10^2 \pm 2.60x10^2$

Table 7 Distribution of different bacterial counts in raw camel milk collected from some Governorates in Egypt

	Total bacterial count c.f.u/ml		Total *Enterobactriaceae* count c.f.u/ml		Total *coliform* count c.f.u/ml		Total *staphylococcal* count c.f.u/ml		Total *staph.aureus* count c.f.u/ml	
	103<106	106<109	102< 104	104< 106	10 < 104	104 < 107	1 < 103	103< 106	1 < 103	103< 105
No. of positive samples	17	133	24	53	23	46	13	44	11	11
%	11	89	16	35	15	31	9	29	7	7

Conclusion

The results herein indicated that camel milk had inferior microbiological quality due to its high contents of total bacterial counts, total *coliform*, total *Enterobacteriaceae* and *staphylococcus spp*. which are not in conformity with the official standards. These pathogenic germs can endanger the keeping quality and safety of raw camel milk. There is a pressing need to improve the hygienic condition and providing adequate sanitary measures from the stage of production till milk consumption. On the other side, food safety education should be raised to camel milk producers, handlers and consumers with a recommendation of pasteurization of consumed raw camel milk.

Ethics

The work did not involve experimental animals or human subjects. As such it was exempted from institutional ethical clearance

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Conflicts of interest

Author declares there is no conflict of interest.

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