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The Hygienic Quality of Raw Milk of Different Species

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Abstract: ***In order to investigate the presence of the bacterial and fungal cells in the locally produced raw milk samples leading to the serious spoilage and public health hazards, a total of 100 raw milk samples were collected randomly at weekly intervals (5 samples/ week) from the milk cans of cows, ewes, buffaloes, goats and camels located inside the farmer's homes in the rural areas of the Wasit province during two climate periods where the first was summer period that extended from the beginning of July to the end of September 2016 while the second was winter period that extended from the beginning of December 2016 to the end of February 2017 (10 samples/ animal species/ season). The microbiological laboratory studies of the cultural investigation during the two climatic periods revealed that there were non-significant (P >0.05) differences in the percentages of confirmed positive results for the presence of aerobic bacterial; coliforms, psychrotrophs and both yeasts and molds between the five types of raw milk samples that belonged to the five different animal species where similar findings of the prevalence levels of contamination (100 %) with the above mentioned organisms were found in the raw milk samples of all the five different animal species during each climatic period. An overall conclusion on the bases of this investigation pointed out that the relatively unhygienic practices and poor sanitation techniques in the milking process by the farmers in the rural areas were reflected on the highest significant (P > 0.05) bacterial and fungal counts in the milk samples of cows, ewes, buffaloes and goats for each season, in comparison to the camel's raw milk samples that had significantly (P > 0.05) the lowest counts for each season. The results that obtained from the current study established the statistically significant (P <0.05) influence of season on the total bacterial and fungal cells counts where all the raw milk samples that were collected from cows, ewes, buffaloes, goats and camels had significantly (P < 0.05). The highest bacterial and fungal cells count during the summer period and the lowest counts during the winter period.***

Keywords: Raw milk, Total bacterial count, Total coliform count, Total yeasts and molds count

1. Introduction

Milk is a vital source of food for humans and animals. Milk is made up of 87% water and the rest is total solids in stable formula and suitable for growth of the human and animal body as well as containing immunoglobulins that act as a defensive means for newborns (1). (2) Reported that cells inside the mammary glands produced milk and it's almost sterile when secreted in udder into the alveoli. After this phase of milk let down, milk contamination with bacteria can occurs from within the udder, outside the udder, and from the surface of tools used for milk handling and storage. Cow's health, surroundings, milking procedures and utensils cleanness can encourage the stages of microbial contamination of the raw milk. Similarly significant are the field temperature of milk and the stretch of time milk is kept before testing and processing that encouragement bacterial growth. Altogether these aspects will assistance the total bacteria counts and the kinds of bacteria present in raw milk bulktank. To determine milk quality microbial testing, besides additional tests for example fat ratio, protein ratio and testing for antibiotics are very essential not only to the producer/processor, but also to the purchaser. The colloidal nature of cow's milk is an important structural feature that affects the end product value in addition to its processing performance (3). The present study aims were: giving a clear picture about the bacterial load of raw milk of cows, ewes, buffaloes, goats and camels before application of sanitary practices, in the production and storage of milk, and investigating the causes of the low quality raw milk and to develop the scientific proposals that can lead to improve the quality of milk.

2. Materials and Methods

This study spanned from July (2016) to February (2017), the total number of milk samples were (100) from five different animals species (cows, ewes, buffalos, goats and camels) in two climatic period (summer period and winter period). All samples were tested by microbiological tests and milk analyzer**.**

3. Microbiological Methods

Ten decimal serial dilutions (10^{-1} to 10^{-7}) for each raw milk sample were prepared in sterile 0.1 % (wt/v) peptone water as a diluent and then pour plated in duplicate for each dilution on to nutrient agar at 45°C for both the total aerobic bacterial counts and psychrotrophic counts, i.e. one milliliter from each dilution was transferred to petri dish and mixed with 15 ml of nutrient agar at 45°C.The total aerobic bacterial colonies were enumerated after aerobic incubation at 37°C for 48 hours while the psychrotrophic colonies were enumerated after aerobic incubation 7°C for 10 days.The total coliforms counts were done by preparing a tenfold decimal serial dilutions(10^{-1} to 10^{-5}) for each milk sample in sterile peptone water 0.1%(wt/v) as a diluent and then pour plated in duplicate for each dilution with violet red bile agar (VRBA). One milliliter of each decimal dilution was transferred into sterile petri dish and mixed with 10 ml of violet red bile agar at 45°C. The mixture was allowed to solidify for 5-10 minutes on a level surface, then additional 5 ml of VRB agar were added as an overlay that completely covering the surface of the solidified medium to inhibit surface colony formation. The coliform colonies were enumerated after aerobic incubation for 24 hours at 32°C (4).Tenfold serial dilution (10-1 to 10-7) for each raw milk

sample were prepared in sterile 0.1% (wt/v) peptone water as diluent and then pour plated in duplicate for each dilution onto OGYE agar and malt agar. The yeasts and molds colonies were enumerated after aerobic incubation at 25°C for 5-7 days. Plates that had 15-150 colonies were selected for counting using the colony counter with magnifying lens (5).

Raw milk constituents and its physical properties

Instrumental analysis: Milkoscan was used to analyze the main components of milk samples such as fat%, SNF%, protein%, lactose%, freezing point and Specific gravity.

PH: The pH of milk sampleswas measured in vitro using electronic PH meter.

Total aerobic bacterial counts

Data revealed that there was a significant ($P < 0.05$) seasonal variation in the mean log values of the total viable aerobic bacterial counts in the raw milk samples for each animal species where all the raw milk samples that were collected from cows, ewes, buffaloes, goats and camels had significantly ($P < 0.05$) lower counts in winter (7.85, 7.84, 7.93, 7.78 and 7.51) log cfu/ml respectively than in summer period (8.84, 8.45, 8.36, 8.33 and 8.00) log cfu/ml respectivelyas shown in Table (1)

Table 1: Total bacterial count of samples during summer and winter period

Source of milk samples	No. of examined samples per season	No. of positive samples per season	Microbial counts (logcfu/ml)	
			summer	winter
			Mean ±SE	Mean ±SE
Cows	10	10	8.84±0.01 Aa	7.85±0.01 Ba
Ewes	10	10	8.45±0.02 Ab	7.84±0.04 Ba
Buffaloes	10	10	8.36±0.03 Ab	7.93±0.04 Ba
Goats	10	10	8.33±0.02 Ab	7.78±0.04 Ba
Camels	10	10	8.00±0.01 A	7.51±0.12 Bb
LSD	0.1694			

∗ Horizontal different capital letters revealed significant ($P < 0.05$) differences between seasons.

∗ Different small letters in a column revealed significant ($P < 0.05$) differences between animals.

Total coliforms counts

In Table(2)The results established the statistically significant ($P < 0.05$) influence of the season on the total coliforms counts in all the five different types of raw milk samples, where all the raw milk samples that were collected from cows, ewes, buffaloes, goats and camels had significantly ($P < 0.05$) higher counts in summer period (7.59. 7.55, 7.50, 7.47 and 7.00 log cfu/ ml respectively) than in winter period (7.11, 7.20, 7.15, 7.13 and 6.76 log cfu/ml respectively).

Table 2: Total coliforms counts of samples during summer and winter period

Source of milk samples	No. of examined samples per season	No. of positive samples per season	Microbial counts (logcfu/ml)	
			Summer	Winter
			Mean ±SE	Mean ±SE
Cows	10	10	7.59±0.04 Aa	7.11±0.01 Ba
Ewes	10	10	7.55±0.04 Aa	7.20±0.02 Ba
Buffaloes	10	10	7.50±0.07 Aa	7.15±0.04 Ba
Goats	10	10	7.47±0.07 Aa	7.13±0.01 Ba
Camels	10	10	7.00±0.07 Ab	6.76±0.07 Bb

∗Horizontal different capital letters revealed significant ($P < 0.05$) differences between seasons.

∗Different small letters in a column revealed significant ($P < 0.05$) differences between animals.

Total psychrotrophic counts

The results that obtained from the current study established the statically significant ($P < 0.05$) influence of the season on the total psychrotrophic bacterial counts in all the five different types of raw milk sample that were collected from cows, ewes, buffaloes, goats and camels which had significantly ($P < 0.05$) the highest counts during the summer period (7.34, 7.26, 7.24, 7.24 and 6.95 log cfu/ ml respectively) and had significantly ($P < 0.05$) the lowest counts during the winter period (6.59, 6.54, 6.57, 6.44 and 5.59 log cfu/ml respectively) as listed in Table(3).

Table 3: Total psychrotrophic counts of samples during summer and winter period.

Source of milk samples	No. of examined samples per season	No. of positive samples per season	Microbial counts (logcfu/ml)	
			summer	winter
			Mean±SE	Mean±SE
Cows	10	10	7.34±0.03 Aa	6.59±0.06 Ba
Ewes	10	10	7.26±0.02 Aa	6.54±0.06 Ba
Buffaloes	10	10	7.24±0.02 Aa	6.57±0.10 Ba
Goats	10	10	7.24±0.02 Aa	6.44±0.02 Ba
Camels	10	10	6.95±0.03 Ab	5.59±0.02 Bb
LSD	0.1942			

∗Horizontal different capital letters revealed significant ($P < 0.05$) differences between seasons.

∗Different small letters in a column revealed significant ($P < 0.05$) differences between animals.

Total yeast and molds counts

In Table(4) the result that obtained from the current study established the statistically significant ($P< 0.05$) influence of the season on the total yeasts and molds counts in all the five different types of raw milk samples, where all the raw milk sample that were collected from cows, ewes, buffaloes, goats and camels had significantly ($P < 0.05$) the highest counts during the summer period (6.50, 6.60, 6.55, 6.40 and 6.10 log cfu/ ml respectively) while had significantly ($P < 0.05$) the lowest counts during the winter period (6.20, 6.21, 6.29, 6.16 and 5.80 log cfu/ml respectively).

Table 4: Total yeasts and molds counts of samples during summer and winter period

Source of milk samples	No. of examined samples Per season	No. of positive samples Per season	Microbial counts (logcfu/ml)	
			summer	winter
			Mean ±SE	Mean ±SE
Cows	10	10	7.34±0.03 Aa	6.59±0.06 Ba
Ewes	10	10	7.26±0.02 Aa	6.54±0.06 Ba
Buffaloes	10	10	7.24±0.02 Aa	6.57±0.10 Ba
Goats	10	10	7.24±0.02 Aa	6.44±0.02 Ba
Camels	10	10	6.95±0.03 Ab	5.59±0.02 Bb
LSD	0.1942			

∗ Horizontal different capital letters revealed significant ($P < 0.05$) differences between seasons.

∗ Different small letters in a column revealed significant ($P < 0.05$) differences between animals.

Raw milk constituents and its physical properties

In Table (5)the average percentages of protein in cow's, ewe's, buffalo's, goat's and camel's raw milk samples were (3.33 %, 3.79 %, 3.82 %, 3.22 % and 2.61 % respectively) where the camel's raw milk samples had significantly ($P < 0.05$) the lowest protein percentage.The lactose percentages in the above mentioned animal species were (4.53 %, 4.53%, 4.96 %, 4.11 % and 3.88 % respectively) where the camel's raw milk samples had significantly ($P < 0.05$) the lowest lactose percentage. The fat percentages in the above mentioned animal species were (3.44 %, 7.07 %, 7.81 %, 3.98 % and 4.48 % respectively) where both the ewe's and buffalo's raw milk samples had significantly ($P <0.05$) the highest fat percentages.The result established the statistically non-significant ($P > 0.05$) differencesinthe percentages of ash between the milk samples of the five different animal species.

Table 5: Raw milk constituents

Source of milk samples	No. of examined samples	Milk composition in %			
		Protein	Lactose	Fat	Ash
Cows	10	3.33±0.18 ab	4.53±0.19 ab	3.44±0.18 c	0.69±0.02 a
Ewes	10	3.79±0.18 a	4.53±0.20 ab	7.07±0.12 a	0.74±0.04 a
Buffalos	10	3.82±0.22 a	4.96±0.04 a	7.81±0.29 a	0.74±0.009 a
Goats	10	3.22±0.19 b	4.11±0.06 ac	3.98±0.16 bc	0.75±0.02 a
Camels	10	2.61±0.12 c	3.88±0.34 c	4.48±0.40 b	0.68±0.02 a
LSD		0.5545	0.5969	0.7588	0.0834

∗Different small letters in a column revealed significant ($P < 0.05$) differences between animals.

Physical properties

From the results obtained in the current study, the average values of specific gravity in the cow's, ewe's, buffalo's, goat's and camel's raw milk samples were (1.026, 1.027, 1.031, 1.024 and 1.029 respectively) where the buffalo's raw milk samples had significantly ($P < 0.05$) the highest specific gravity value. The average values of the freezing points in the above mentioned five different animal species were (-0.51, -0.50, -0.57, -0.51and -0.53 respectively) where the buffalo's raw milk samples had significantly ($P < 0.05$) the lowest freezing point value. The average PH values in the above mentioned five different animal species were (6.58, 6.96, 6.40, 6.70 and 6.62 respectively) where the ewe's raw milk samples had significantly the highest PH value as listed in Table(6).

Table 6: Physical properties of raw milk

Source of milk samples	No. of examined samples	Specific gravity	Freezing point	PH
Cows	10	1.0260±0.0002 bc	-0.510±0.02 ab	6.58±0.12 b
Ewes	10	1.0276±0.0007 b	-0.500±0.007 a	6.96±0.10 a
Buffalos	10	1.0318±0.0002 a	-0.568±0.01 b	6.40±0.08 b
Goats	10	1.0247±0.0005 c	-0.511±0.009 ab	6.70±0.15 ab
Camels	10	1.0299±0.003	-0.529±0.03	6.62±0.08 b
		a	ab	
LSD		0.0022	0.0627	0.3296

∗Different small letters in a column revealed significant ($P < 0.05$) differences between animals.

4. Discussions

Total bacterial counts

The total aerobic bacterial counts is used as an indicator for the application of the hygienic conditions and the safety of milk and other dairy products (6).The diary processing plants in the USA required that the total aerobic bacterial counts in the raw milk leaving the dairy farm was < 100.000 cfu/ml and that in commingled milk at the processing plant was < 300.000 cfu/ml (7).High warm storage temperature of the raw milk inside the milk cans during the summer period was regarded as good reason for encouraging the growth and multiplication of all kinds of microorganisms (8). The results of the current study were in agreement with (9) and also in consistent with(10) where they established the statistically significant influence of the season on the total microbial counts in the raw milk samples. Also it has been suggested that the microbial seasonality could be related to the abundance of flies in the summer period which act as a mechanical vectors.The lowest mean log values of total bacterial counts were reported in camel's milk in both season in summer and winter were (8.00, 7.51 log cfu/ ml respectively) this result was in agreement with (11) who showed that camel's milk possesses antibacterial and antiviral activities and they suggested that this milk contains protective proteins which may have possible role for enhancing immune defense mechanism.

Total Coliforms counts

The genera Escherichia, Klebsiella, Enterobacter, Serratia, and Citrobacter are collectively called the Coliform bacilli and some of them are opportunistic pathogens responsible for a wide range of infections, but many species are members of the normal intestinal flora (12). According to (13) coliforms bacteria are used as indicators of sanitations during the milk production process.In this study, the coliforms counts was so high in the raw milk samples that were collected from all the five different animal species as illustrated in Table (2) in both summer and winter periods and such result was in agreement with (14) who noted that coliforms counts above 500 cfu/ml indicated poor hygienic practices during equipment cleaning or between milking with common contaminants such as bedding, manure, soil or water.

Total psychrotrophic counts:

Psychrotrophicmicroorganisms represent a substantial percentage of the bacteria in raw milk, with pseudomonads and related aerobic, Gram-negative, rod-shaped bacteria being the predominant groups. Typically, 65–70% of the psychrotrophs isolated from raw milk samples were Pseudomonas species (15) (16). The testing of psychortrophic bacteria was very important because these bacteria were able to produce heat stable proteases and lipases that even at refrigerated storage conditions reduced the shelf life of the fluid milk (17).The results of the current study revealed that there was a high psychrotrophic counts in the raw milk samples which was in agreement with(18)

who reported that Psychrotrophs comprised the largest percentage of bacteria in milk and caused spoilage in refrigerator temperatures at or below 7°C also was in agreement with(19) who reported that the presence of high numbers of Gram-negative bacteria in milk was noted in situations of poor hygienic standards and generally reflected poor udder preparation, poor sanitation or deficiencies with respect to the hygiene of equipment.

Total yeasts and molds counts

Fungal cells are widely distributed in the dairy herds environment and can be considered as a part of the normal flora of the food products. The presence of yeasts and molds in the raw milk is objectionable because they can grow at a wide range of the environmental temperature and PH values and their counts are used as an index for the proper sanitation and the quality control of the milk (20).The obtained results were nearly similar to those obtained by the(21) who reported a significant ($P < 0.05$) decrease of yeasts and molds counts in all the milk samples in the winter season in comparison to the summer season.

Physical analysis of milk samples

The fat% in the cow's and ewe's raw milk samples (Table 5) were in agreement with (22) but in disagreement with the fat% of both goat's and camel's raw milk samples who reported that their fat% were 3.90% and 3.6% respectively.The fat contents in ewe's and Buffalo's raw milk samples were higher than that in the cow's, goat's and camel's raw milk (Table 4.12) and these findings were online with those results that reported by(23).The results of total solids (TS) % and solids non-fat (SNF)% percentages were lower than that in the normal cow's milk and this result was agreement with (24) who noted that the (TS%) and the (SNF%) at the markets milk were extensively subjected to malpractices such as adulteration with water which were probably carried out during the handling of the milk starting from the milking till it reached the consumers.The density of the cow's milk was 1.026 which was lower than the standard density of the cow's raw milk that should be within the range of 1.028 to 1.036. The density of cow's , ewe's and buffalo's raw milk samples as shown in Table (6) were lower than the standards and such results could be attributed to the commercial adulteration by added water which was in agreement with (25) and (26) who noted that adulteration with extraneous water in milk apparently decreased the relative density.

5. Conclusions

After analyzing all the data we can conclude that the raw milk quality in wasit province needs to be improved and mainly in the microbial sense. The result of the present study confirmed that the high levels of milk contamination with the aerobic bacteria, coliforms, psychrotrophs and both yeasts and molds reflected the relatively neglected hygienic practices with poor sanitation technique in the milk production in Wasit province. The total aerobic bacterial counts were far too high when compared to the Iraqi, Egyptian and European standards. For processors to be able to manufacture a fluid milk that can satisfy the consumers in regards to an acceptable flavor and shelf life requires, more care in the milking process, handling, storage and distributions.

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