

Research article

Comparative study of some indicator bacteria of sheep and goats raw milk in Al-Qadisiyah Province

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(Received 19/7/2017, Accepted 25/10/2017)

Abstract

*The present study was carried out in Al-Diwaniyah city and its different districts, which includes (city center, Afak district and their regions, Al-Hamza district and their regions, Al-Shamiya district and their regions, Al-Daghra regions and its villages) during a period November 2009 to July 2010. A total of samples used in this study were 120 raw milk samples collected includes 60 samples for each species for (sheep and goats) for two different season of year (Winter and Summer), first start from (beginning November 2009 to end of the January 2010), and the second start from (beginning May to July 2010) The results of this study revealed the rising the averages of microbes contaminated raw milk samples in each species of animals included in this study where raw milk samples for sheep recorded (0.16×10^6 CFU/ml) and goats (0.13×10^6 CFU/ml). The highest averages of total Coliform bacterial count were found in raw milk samples of sheep and goats (0.15×10^6 CFU/ml). While averages of *Staphylococcus aureus* were high in raw milk samples of sheep (0.20×10^6 CFU/ml) and less in raw milk samples of goats (0.14×10^6 CFU/ml). While the average of *Streptococcus spp.* were (0.30×10^6 CFU/ml) and (0.27×10^6 CFU/ml) for sheep and goats respectively. Seasons of the year had effect on average of total bacterial count studied for each samples of raw milk for all species of animals included in this study. Where shown rising averages of all microbes numbers during summer period while there are decreasing in average of microbes during winter period which variances significantly ($P \leq 0.05$).*

Keywords: Comparative study, Indicator bacteria, Raw milk, Sheep, goats

Introduction

Historically, milk has been a staple food for various layers of population. It is a rich natural food and is characterized by easy to prepare for consumption and taste by consumers in addition to being easy to digest so preferably in all stage of life, In general raw milk provides the body with its nutrient needs in a balanced manner and compensates for deficiencies in other foods. The average daily human consumption of milk and milk products in most of the world has become a measure of progress (1, 2). The milk extracted from the lactic gland is free from

bacteria but is quickly contaminated by two main sources, namely natural flora in the channels that pass through the milk inside the udder and the bacteria that are derived from the external animal environment such as the hands of the milkers, milk machine and animal body cover (3). Because of importance of sheep and goats milk in local food in the city of Al-Diwaniyah. Therefore, this study was designed to investigate the extent of the presence of some contaminated bacteria and identify the total numbers and

the effect of winter and summer on total numbers of bacteria.

Material and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 428

Sample collection

Milk samples were collected after the milk with properly homogenized the samples were carried at temperatures below 5°C where they were placed in refrigerated containers. So that the bottleneck of the milk sample is out of the ice until it reaches the laboratory and then the bacterial tests were carried out within a period not exceeding three hours. Chemicals and Instruments media were prepared as described by manufacturer's instructions and synthetic Media prepared according to what mentioned in (4, 5, 6).

Bacterial count

After collecting, the milk samples and transferring to the laboratory inoculated directly on the Petri dishes containing the following media:

- 1- Nutrient agar
- 2- MacConkey agar
- 3- Mannitol salt agar
- 4- Blood agar

The dishes were incubated after 37°C bacterial transplantation for 24-48 hours and the developing colonies were examined on these cultures to determine the size and shape of the colonies swabs were made from the colonies and stained in a gram stain the bacterial colonies were then examined and the biochemical tests of the isolated bacteria were carried out by each type and the standard count method (SPC) used to examine the raw milk as described in (7, 8, 3)

A-Total bacterial count

Nutrient agar was used to calculate the number of live bacteria and the way they were spread to dishes and incubate at 37°C for 24 hours

B-Coliform bacteria

Coliform bacteria colonies that appear on the MacConkey agar and as dry pink colonies on the center of EMB, whose colonies appeared in metallic shiny color and long stick a microscope that appeared in long

C-*Staphylococcus aureus*

Used the salt mannitol agar and the incubated at 37°C for 24 hours (4). The colonies appeared circular, convex, shiny, translucent, and smooth and with circular edges that were not serrated and resembled drops of oil paint and color.

D-*Streptococcus spp*

The colonies were circular white to gray-colored transparent alpha and beta blood hemolysis and were negative for catalase, oxidase and a microscopically examination was a positive form of a spherical or oval spherical shape

E-bacterial diagnosis

The diagnosis of isolated bacteria was based on the following principles: characteristics of colonies the characteristics of color, size and shape included the presence of a transparent region around the colony. The blood decomposition pattern on the lactose fermentation medium on the MacConkey center and the mannitol fermentation on the mannitol agar medium (9, 10, 4)

F-Biochemical tests (11, 4)

G-Maintenance of bacterial colonies

Samples were extracted using agar of brain and heart infusion. The tubes were vaccinated with bacterial isolates and incubated at 37 °C for 24 hours. The isolates were then kept in the refrigerator. The monthly maintenance of the isolates was carried out by renewing their cultivation on the brain and heart diffusion broth to stimulate isolation before transplantation (4)

H-Statistical analysis

All experiments and data were presented analysis as mean \pm standard error Analysis was done in Statistical Package for Social Science SPSS program some Experimental data were analyzed by CRD and Chi-square

P value ≤ 0.005 was considered as statistically significant (12,13,14)

Results

A-Total bacterial count

The results showed that the total number of bacteria in raw milk samples not recorded significant variances between experimented animals in sheep were 0.16×10^6 CFU/ml while in goats raw milk were 0.13×10^6 CFU/ml table (1) and during the winter and summer period significant variances in each animals ($P \leq 0.05$) were in sheep 0.14×10^6 CFU/ml and 0.13×10^6 CFU/ml and in goats 0.17×10^6 CFU/ml and 0.12×10^6 CFU/ml during summer and winter period respectively table(2).

B-Coliform bacteria

Table (1) shows no variances between raw milk of experimented animals with total coliform bacteria which recorded 0.15×10^6 CFU/ml in sheep and 0.15×10^6 CFU/ml in goats, whereas during winter and summer they reached 0.12×10^6 CFU/ml and 0.09×10^6 CFU/ml in sheep milk respectively and recorded 0.13×10^6 CFU/ml and 0.22×10^6 CFU/ml in goats respectively with significant variance ($P \leq 0.05$) Table (2).

C-Staphylococcus aureas

The results of the statistical analysis showed a significant difference ($P \leq 0.05$) in the total number of *staphylococcus aureas*

between the raw milk samples of sheep and goats were 0.20×10^6 CFU/ml and 0.14×10^6 CFU/ml respectively. The results of the statistical analysis showed a significant increase ($P \leq 0.05$) in the number of these bacteria during winter and summer period in raw milk samples for sheep and goats table (2) which recorded (0.12×10^6 CFU/ml, 0.09×10^6 CFU/ml) and (0.13×10^6 CFU/ml, 0.15×10^6 CFU/ml) respectively.

D-Streptococcal spp.

The results of the study showed that the total number of streptococcal spp. in the raw milk samples for the sheep were 0.30×10^6 CFU/ml and in goats 0.27×10^6 CFU/ml with significant variances table (1). Whereas The number during the winter and summer periods were (0.11×10^6 CFU/ml, 0.10×10^6 CFU/ml) and (0.36×10^6 CFU/ml, 0.39×10^6 CFU/ml) respectively with significant variances ($P \leq 0.05$) Table (2).

E-Bacterial isolation and diagnosis

Biochemical tests were carried out on isolates of microorganisms obtained during the study. The results showed no significant variance numbers of *staphylococcus aureas* and *streptococcus spp.* Isolates while recorded highly significant ($P \leq 0.05$) with coliform bacteria.

Table (1): total number of bacteria \pm SE

animals	Total bacterial count \pm SE $\times 10^6$	Coliform count \pm SE $\times 10^6$	Staphylococcus count \pm SE $\times 10^6$	Streptococcus count \pm SE $\times 10^6$
Sheep	0.16 \pm 44 A	0.15 \pm 4.4A	0.20 \pm 0.50 A	0.30 \pm 0.29 A
Goats	0.13 \pm 47A	0.15 \pm 4.0 A	0.14 \pm 0.44B	0.27 \pm 0.33 B

Different litters mean significant variances ($P \leq 0.05$) in same column

Table (2) total number of bacteria \pm SE during season periods

animals	season	Total bacterial count \pm SE $\times 10^6$	Coliform count \pm SE $\times 10^6$	Staphylococcus count \pm SE $\times 10^6$	Streptococcus count \pm SE $\times 10^6$
Sheep	winter	0.14 \pm 40 A	0.22 \pm 4.6 A	0.12 \pm 0.35 A	0.11 \pm 24 A
	summer	0.13 \pm 49 B	0.19 \pm 4.2 A	0.09 \pm 0.64 B	0.10 \pm 0.33 B
Goats	Winter	0.17 \pm 41 A	0.13 \pm 3.3 B	0.13 \pm 0.36 A	0.36 \pm 0.28A
	summer	0.12 \pm 53 B	0.22 \pm 4.6 A	0.15 \pm 0.52 B	0.39 \pm 0.37B

Different litters mean significant variances ($P \leq 0.05$) in same column

Table (3): numbers and percent of isolates

		Coliform	Staphylococcus	Streptococcus
Sheep	No.of isolates	21	15	10
	%	42	30	20
Goats	No.of isolates	19	13	11
	%	39.5	27	22.9

Discussion

A-Total bacterial count

The results showed that the total number of bacteria in the raw milk samples of the study animals exceeded the permissible limits. The samples of sheep and goats recorded highly number of bacterial counts, these numbers are due to failure to follow the health instructions in animal management and cleanliness The environment surrounding it, the cleanliness of the utensils used to collect and store milk, the low interest of milking operators, the high pollution surrounding the storage of milk from flies and rodents, and the poor handling and transport that often lack cooling until reaching consumers. Consistent with the findings (15). The high number of total Bacteria during summer period is due to the fact that the temperature during the summer is more suitable for the growth and multiplication of bacteria especially if it is close to the optimum degree of growth in addition to the increased risk of contamination of water used for drinking and washing milk utensils which adds bacteria to the bacteria remaining from the previous ring (16, 17, 18, 19). Their results generally indicate that during the winter season, the rates are lower than in the summer season. Summer heat allows the growth of coliform bacteria and heat-loving bacteria in milking tools, especially under the conditions of improper sterilization of milking tools and machines.

B-Coliform bacteria

The results of the total coliform bacteria showed that they exceeded the health limits, which should not exceed 1×10^1 CFU/ml in raw milk (20). And these numbers are due to fecal contamination from the wool and hair of the animal and the hands of the young

people who do not meet the conditions of health and personal hygiene as well as refrigeration of raw milk after production and this is consistent with what indicated (15,16). The presence of coliform bacteria in raw milk is an indicator of direct fecal contamination or The results were consistent with the results of (15). The season also had an effect on the increase in the total number of coliform bacteria in the raw milk samples of sheep and goats during the summer than in winter. Higher during the summer and is often washed by the water of the river almost stagnant and contaminated with bacteria raising the rate in the summer period for winter and this is consistent with the findings of (21, 22, 23).

C-*Staphylococcus aureus*

It is noted that the rates of the number of *Staphylococcus aureus* are high to the extent that they may pose a danger to public health because they are related to cases of food poisoning after the consumption of contaminated raw milk as 5×10^5 CFU/ ml to 1×10^7 CFU/ ml units formed for the Status of food poisoning. The high number of staphylococcus aureus in all samples of raw milk for study animals is due to the wide spread of these bacteria in nature as well as the presence of animal skin, wool, hair and wet places of the body such as the nose and mouth milk is also an ideal medium for the growth of *Staphylococcus aureus* and the production of toxins because of its high humidity and nutrients The pH function is ideal and this corresponds to what is indicated by (24, 25, 26). The rates were higher during the summer months than the winter months in the raw milk samples collected from the Al-Diwaniyah markets

and there was also an increase in summer season rates than winter season.

D-Streptococcus spp.

The results of the study also showed that the rates of Streptococcus spp. were high in all raw milk samples of the study animals, this high pollution is dangerous because it causes the food poisoning of the raw milk consumers contaminated with these bacteria and just isolating them from any food is an indicator. The high pollution of these bacteria is caused by unclean pots and animal feed, especially in the form of cages, and bacteria are the main causes of mastitis in farm animals. These results are consistent with those recorded (27, 28, 29, 30, 15). While significantly lower in the raw milk samples collected from the Czech Republic where the rate was 1×10^3 CFU/ml attributed this to the application of the conditions of health in the

process of milking mechanism and the cleanliness of workers and means of transport of cooled and clean milk. The period of study impact on the variability of the rates of bacteria the summer season played a role in raising the rate of bacteria in raw milk for (23).

E-Isolation and bacterial diagnosis

The variation observed in the types and number of bacterial isolates in the current study compared with other studies is due to different conditions and places of study. The animal environment and management have an important role in determining the type of isolates and their number in the raw milk samples of the study animals as well according to the different conditions of milk production, either manually to the extent of compliance with the sanitary conditions of production (31, 32, 33).

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