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Evaluation of the Hygienic Quality of Market Milk of Khartoum State (Sudan)

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Abstract: This study was carried out between August 2003 and March 2004. One hundred and twenty milk samples were collected from supermarkets in Khartoum State. Sixty milk samples were collected during summer and other 60 during winter from the same supermarkets. The milk samples were examined for microbial quality that include total bacterial count, spore forming bacteria count, coliform count. Enumeration, isolation and identification of *E. coli*, *S. aureus*, *Salmonella* spp. and the presence of Brucella as detected by milk ring test were also estimated. The criteria used for identification of the isolates were reaction to Gram's stain, morphological appearance, cultural characteristics and biochemical tests. Similarly phosphatase test was done. The present study revealed that high average total bacterial count ($5.63\times10^{9}\pm2.87\times10^{10}$ cfu mL^{-1}) was found for the raw milk samples. Moreover, during summer the total bacterial count of milk ($1.04\times10^{10}\pm4.01\times10^{10}$ cfu mL^{-1}) was higher than during winter ($9\times10^{8}\pm2.51\times10^{9}$ cfu mL^{-1}). *Staphylococcus aureus* was detected in 46.7% of the milk samples with mean count of $4.9\times10^{4}\pm1.29\times10^{6}$ cfu mL^{-1}. During summer the mean count was $7.44\times10^{5}\pm1.66\times10^{6}$ cfu mL^{-1}, which was higher than the count during winter ($1.61\times10^{5}\pm2.3\times10^{5}$ cfu mL^{-1}). *Escherichia coli* were detected in 2.5% of the milk samples with a mean of $6.0\times10^{5}\pm7.94\times10^{5}$ cfu mL^{-1} and coliforms bacteria were detected in 82.5% with a mean of $3.32\times10^{6}\pm1.43\times10^{7}$ cfu mL^{-1}. Also during summer the mean was $5.15\times10^{6}\pm2\times10^{7}$ cfu mL^{-1}, which was higher than during winter ($1.45\times10^{6}\pm3\times10^{6}$ cfu mL^{-1}). Spore forming bacteria were detected in 32.5% of the milk samples with a mean of $4.81\times10^{6}\pm1.4\times10^{7}$ cfu mL^{-1}. Also during summer the mean was $7.15\times10^{6}\pm1.79\times10^{7}$ cfu mL^{-1}, was higher than during winter ($1.45\times10^{6}\pm3\times10^{6}$ cfu mL^{-1}). There was no *Salmonella* spp. in any of the milk samples collected during the present study, while milk ring test for Brucella showed that 44.1% of the milk samples were positive for Brucella. Moreover 54.4% of which were detected during winter and 45.61% were found during summer. Also during this study 44.17% of the milk samples were positive to phosphtase test. All the milk samples (100%) were found to be negative to the presence of formalin. Conclusions and recommendations for improvement of hygienic quality of marketed milk in Khartoum State were suggested. Sanitary standards should be established in this country so as to control milk production and marketing. Further studies should be done to evaluate safe and good supply of consumed milk.

Key words: Hygine, quality, pathogens, market, mick, Khartoum State (Sudan)

Introduction

Hygienic controls in raw milk are that the milk should be obtained from healthy animals and from animals not been treated with antibiotics or other veterinary drugs, which can be transferred to the milk

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(Murphy and Boor, 2000). Similarly milking routines should prevent the damage of tissues, which lead to infections of the udder and prevention of the contamination of milk by the stable environment and milking equipment as well as controlling temperature and time in order to minimize the growth of pathogens (IDF, 1994).

Measurement of bacterial numbers in milk is of interest because they are indicator of poor hygienic production or ineffective pasteurization of milk (Harding, 1999). Gran (2002) concluded that hygienic aspects are linked with transportation, preservation and handling of dairy product.

In Brazil, Beloti *et al*. (1999) mentioned an advertising campaign involved public health department was developed to stop the sale of unpasteurized raw milk and to show its risk because microbial studies revealed 4-7 positive samples in Brucella ring test out of 42 milk samples.

Marketing of milk and milk products is of greatest importance since successful operation of many farms is dependent upon the income from the milk sold, particularly when dairying is the major enterprise (El Aggab, 1996). Mustafa (1994) mentioned that the quantities that go into shops of Khartoum State in the morning are refrigerated for short time before selling, however refrigeration is a service of high efficiency in milk market system in Khartoum State and this service is not available for producers and for many selling centers which often result in losses due to perishability of the product.

In Sudan many researchers reported on the importance of milk in public health and stated that because of prevalence of some communicable diseases of milk and spoilage of this perishable product sanitary standards regulating milk production and marketing, should be established in Sudan and setting plans for improvement of milk hygiene and quality (Mustafa and Idris, 1976; Mohamed *et al*., 1999; Yagoub *et al*., 2005; Elmagli and El-Zubair, 2006). Hence the present study is a contribution to the evaluation the hygienic quality of market raw milk in Khartoum State. Enumeration, isolation and identification of some pathogenic bacteria in the market's milk collected during summer and winter were also estimated.

Materials and Methods

Source of Samples

In this study 120 milk samples were collected from 60 supermarkets in Khartoum State. Sixty sample in summer (August- September 2003) and the other 60 in winter (December 2003-January 2004) at both season twenty milk samples were collected from each big city in Khartoum State.

Collection of Samples

Samples were collected from four shops in each selected area in each city from five supermarkets. The collection was done at the evening in sterilized labeled bottles. They were kept in the refrigerator at 4°C and transported in an ice box to the laboratory of the Department of Dairy Production, Faculty of Animal Production, University of Khartoum.

Microbial Examination of Milk Samples

Microbiological parameters studied include the counts of *E. coli*, *Staphylococcus aureus*, coliforms bacteria, Spore forming bacteria and *Salmonella* spp.

Types of cultural media used include plate count agar (S-D's Product No. 7405) that was used for total bacterial count, Mannitol salt agar (Hi Media M118) a differential and selective medium used for *Staphylococcus aureus*. Eosin methylene blue agar (Oxoid CM69) a selective and differential medium used for *Escherichia coli*. MacConkey's agar (Hi Media M081) was used for detection and enumeration of coliform bacteria. Salmonella-Shigella agar (Hi Media M108) is a selective medium for salmonella and shigella, Sterilization of equipment was done by autoclaving at 121°C for 15 min, while all the media were sterilized by autoclaving at 121°C for 15 min, except MR-VP media, sugar media and S.S agar media (Barrow and Feltham, 1993). Serial dilutions were made according to Richardson (1985). Preparations of samples for total spore count were done according to Harrigan and McCance (1976). The counting and calculation of the colonies were done manually. Isolation and identification were done according to Harrigan and McCance (1976) and Barrow and Feltham (1993).

Milk Ring Test

It is an agglutination reaction used to detect the presence of antibodies of Brucella in the milk. The tetrazolium stained antigen was obtained from the Central Veterinary Research Laboratory, Soba using the method described by Cruickshank *et al*. (1975).

Statistical Analyses

The analysis was carried out using SPSS program (Statistical Package for Social Sciences). All the data of this experiment were analyzed statistically by using complete randomized design and least significant difference test. One way ANOVA was used to determine the effect of season, city and area on milk samples.

Results and Discussion

Results of total bacterial count obtained during the present study showed high bacterial count, which ranged between 1.13×10^{5} to 2.45×10^{11} cfu mL^{-1} (Table 2). During summer the total mean count of market milk samples was $1.04\times10^{10}\pm4.01\times10^{10}$ cfu mL^{-1}, while during winter it was $9\times10^{8}\pm2.51\times10^{9}$ cfu mL^{-1}. This might be due to the fact that high temperature during summer enhances the growth and multiplication of bacteria. This agreed with Aleksieva and Krusher (1981) who stated that microbial contamination correlated strongly with the season and the highest numbers were noted during the warm months. The high bacterial counts for raw milk were expected under tropical condition like Sudan due to the fact that high temperature enhances growth and multiplication of bacteria (Barakat, 1995). Moreover, Dirar (1975) stated that under tropical condition many factors such as high temperature, absence of sanitary conditions for production of milk in the dairy farms and unavailability of cooling during handling and transportation of milk affect the quality of milk. When the milk samples collect from different cities and areas were compared it was observed that there were significant differences ($p\leq 0.01$), which indicated that the quality of market milk differ from city to another (Table 4). The total mean bacterial count of market milk collected from the different cities was found to be $5.63\times10^{9}\pm2.87\times10^{10}$ cfu mL^{-1} (Table 2). This was agreed with Khan *et al.* (2002) who found that the total viable count in raw milk ranged between 1.59×10^{10} to 2.59×10^{11} cfu mL^{-1}. Also it was agreed with Mahari and Gashe (1990) who found that the total viable count was ranged between 4×10^{7} and 1×10^{9} cfu mL^{-1}. Similarly Godefay and Molla (2000) found the mean total aerobic plate count in milk upon arrival at the processing plant was 1.9×10^{8} cfu mL^{-1}. However in Germany Jung (2002) stated that in recent years there has been a reduction in the bacterial content in raw milk due to improvement in milk hygiene. However there has been a significant increase in the milk content of cold tolerant bacteria in the last 10 years, due to an increase in the duration of milk storage and long distance transport of milk as he reported.

It was observed that the milk samples collected from Khartoum North had relatively high mean count ($1.6\times10^{10}\pm4.84\times10^{10}$ cfu mL^{-1}) than those of Khartoum ($5.9\times10^{8}\pm1.28\times10^{9}$ cfu mL^{-1}) and Omdurman ($2.6\times10^{8}\pm8.51\times10^{8}$ cfu mL^{-1}). This might be attributed to the fact that 27 raw milk samples from Khartoum North were phosphatase positive compared to 8 and 18 were positive in Khartoum and Omdurman, respectively (Table 1). Also sanitary conditions for production of milk in

Table 1: Incidences and frequencies of microorganisms in milk samples collected from different supermarkets in Khartoum State

Cities	Areas	*S. aureus*	*E. coli*	Coliform	SFB	*Brucella*	No. of positive phosphtase test samples
Khartoum	Elshabia	9	1	9	8	8	8
North	Hilat hamed	10	1	8	5	9	9
	Eldurushab	4	0	4	10	0	10
	Kouber	10	1	10	7	10	-
	Sub total	33 (58.93%)	3 (2.5%)	31 (31.3%)	30 (76.92%)	27 (50.94%)	27 (76.94%)
Khartoum	Elgerif	0	0	10	0	0	0
	Nasir	4	0	10	3	6	6
	Elkakakla	0	0	8	0	0	0
	Elamarat	1	0	10	0	2	2
	Sub total	5 (8.93%)	0	38 (38.4%)	3 (7.69%)	8 (15.09%)	8 (15.09%)
Omdurman	Beet Elmal	6	0	8	0	4	4
	Elthowra	6	0	6	0	3	3
	Ombeda	0	0	8	2	2	2
	Elfitahab	6	0	8	4	9	9
	Sub total	18 (32.14%)	0	30 (30.3%)	6 (15.39%)	18 (33.96%)	18 (33.96%)
	Total	56 (46.7%)	3 (2.5%)	99 (82.5%)	39 (32.5%)	53 (44.17%)	53 (44.1%)

the farm, unsanitary conditions associated with handling of the samples within supermarkets and inadequate cooling during transportation and storage of milk might be some factors, as indicated by the questionnaire from the marketing centers (not shown data).

The rate of isolation of spore forming bacteria was 32.5% in the three cities (Table 1) with the total mean of $4.81\times10^{6}\pm1.4\times10^{7}$ cfu mL^{-1} (Table 2). This agreed with Shamanova *et al.* (1998) who found that all samples contained aerobic spore forming bacteria with the average value of $9.0\pm0.4\times10^{2}$ cfu cm^{-3}. Similarly Hahn *et al.* (1999) reported that *B. cereus* was detected in 8.1% of the samples. The presence of these organisms in raw milk are often related to poor farming practices for example feeding cows with poor quality silage and inadequate cleaning system (IDF, 1994). Also Giffel *et al.* (2002) reported that silage is considered to be a significant source of contamination of raw milk with spores. The present study showed that there were non significant differences of spore forming bacteria of milk samples collected from different cities, areas and seasons (Table 4). It was also observed that the milk collected from Khartoum North had the highest rate and mean of spore forming bacteria (76.92% and $6.23\times10^{6}\pm1.58\times10^{7}$ cfu mL^{-1}) than that collected from Khartoum (7.69% and $3.08\times10^{5}\pm1.13\times10^{5}$ cfu mL^{-1}) and Omdurman (15.39% and $5.51\times10^{3}\pm5.2\times10^{3}$ cfu mL^{-1}) as shown in Table 2. During summer the total mean of spore forming bacteria in market milk was found to be $7.15\times10^{6}\pm1.79\times10^{7}$ cfu mL^{-1} and during winter it was $1.45\times10^{6}\pm3.0\times10^{6}$ cfu mL^{-1} (Table 2). Also during both seasons the raw milk samples collected from Khartoum North had the highest mean count than that collected from Omdurman and Khartoum.

The total rate of isolation of coliform bacteria was found to be 82.5% (Table 1) and the mean count was $3.32\times10^{6}\pm1.43\times10^{7}$ cfu mL^{-1}. These results were agreed with Al-Taraz *et al.* (2003) who detected the coliform in 142 samples of 160 milk samples and the total coliforms ranged from 2.5×10^{4} to 1.4×10^{6} cfu mL^{-1}. Similarly Aleksieva and Krusher (1981) found that in about 27% of the samples the coliform count was up to 1.4×10^{4} cfu mL^{-1} and in about 42% it was about 1×10^{5} cfu cm^{-3}. However, these results were higher than those of Hassan and Al-Sanjary (1999) who found that the mean fecal coliform counts was 2.1×10^{4} cfu mL^{-1}. Similarly Godefay and Molla (2000) found the mean coliform count was 7.1×10^{4} cfu mL^{-1} upon arrival at the processing plant. Also, Jayarao and Wang (1999) detected coliforms in 62.3% of the bulk milk samples with mean count of 3.4 log. 10 cfu mL^{-1}. However Murphy *et al.* (2001) stated that coliforms are found in fecal and bedding materials and poorly cleaned milk handling and storage equipment. It was observed that when the means of the milk were compared there were non significant differences (Table 4), this might

Table 2: Some microbiological quality of milk samples collected from different cities in Khartoum State during summer and winter seasons

		Total bacterial (cfu mL^{-1})			Coliform (cfu mL^{-1})		
Cities	Seasons	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
Khartoum north	Winter	$2.39\times10^9\pm3.97\times10^9$	1.75×10^5	1.3×10^{10}	$1.55\times10^6\pm3.52\times10^6$	0	1.38×10^4
	Summer	$2.97\times10^{10}\pm6.64\times10^{10}$	2.7×10^8	2.45×10^{11}	$1.19\times10^7\pm3.46\times10^7$	0	1.39×10^8
	Sub total	$1.6\times10^{10}\pm4.84\times10^{10}$	1.75×10^5	2.45×10^{11}	$6.89\times10^6\pm2.52\times10^7$	0	1.39×10^8
Khartoum	Winter	$2.31\times10^8\pm5.31\times10^8$	1.37×10^5	2.4×10^9	$1.91\times10^6\pm3.5\times10^6$	0	1.43×10^7
	Summer	$9.5\times10^8\pm1.67\times10^9$	2.5×10^5	5.5×10^9	$2.88\times10^6\pm3.9\times10^6$	0	1.5×10^7
	Sub total	$5.9\times10^8\pm1.28\times10^9$	1.37×10^5	5.5×10^9	$2.39\times10^6\pm3.69\times10^6$	0	1.5×10^7
Omdurman	Winter	$9.21\times10^7\pm1.97\times10^8$	1.13×10^5	8.88×10^8	$7.54\times10^5\pm1.13\times10^6$	0	3.7×10^6
	Summer	$4.4\times10^8\pm1.18\times10^8$	3.2×10^5	5.35×10^9	$8.42\times10^5\pm1.22\times10^6$	0	4×10^6
	Sub total	$2.6\times10^8\pm8.51\times10^8$	1.13×10^5	5.35×10^9	$7.98\times10^5\pm1.16\times10^6$	5.5×10^3	4×10^6
Sub total	Winter	$9\times10^8\pm2.51\times10^9$	1.13×10^5	1.3×10^{10}	$1.45\times10^6\pm3\times10^6$	4×10^4	1.43×10^4
	Summer	$1.04\times10^{10}\pm4.01\times10^{10}$	2.5×10^5	2.45×10^{11}	$5.15\times10^6\pm2\times10^7$	4.5×10^2	1.39×10^8
	Total	$5.63\times10^9\pm2.87\times10^{10}$	1.13×10^5	2.45×10^{11}	$3.32\times10^6\pm1.43\times10^7$	4×10^2	1.39×10^8

		Spore forming bacteria (cfu mL^{-1})		
Cities	Seasons	Mean±SD	Min.	Max.
Khartoum north	Winter	$1.79\times10^6\pm3.26\times10^6$	0	7.5×10^6
	Summer	$9.62\times10^6\pm2.03\times10^7$	0	8.5×10^7
	Sub total	$6.23\times10^6\pm1.58\times10^7$	0	8.5×10^7
Khartoum	Winter	0	0	0
	Summer	$3.08\times10^5\pm1.13\times10^5$	0	4.25×10^5
	Sub total	$3.08\times10^5\pm1.13\times10^5$	0	4.25×10^5
Omdurman	Winter	$3.7\times10^6\pm4.7\times10^3$	0	8.75×10^3
	Summer	$4.67\times10^3\pm5.62\times10^3$	0	1.25×10^4
	Sub total	$5.51\times10^3\pm5.2\times10^3$	2×10^2	1.25×10^4
Sub total	Winter	$1.45\times10^6\pm3\times10^6$	1×10^2	7.5×10^6
	Summer	$7.15\times10^6\pm3\times1.79\times10^7$	1.5×10^3	8.5×10^7
	Total	$4.81\times10^6\pm1.4\times10^7$	1×10^2	8.5×10^7

M = Mean, SD = Standard Deviation

indicated that there was no different in coliforms numbers in the different cities and areas. Moreover, it was observed that the milk samples collected from Khartoum North had the highest mean coliform count ($6.89\times10^6\pm2.52\times10^7$ cfu mL^{-1}) than that collected from Khartoum ($2.39\times10^6\pm3.69\times10^6$ cfu mL^{-1}) and Omdurman ($7.98\times10^5\pm1.16\times10^6$ cfu mL^{-1}) as shown in Table 2. During summer the mean count was $5.15\times10^6\pm2\times10^7$ cfu mL^{-1} and during winter it was $1.45\times10^6\pm3\times10^6$ cfu mL^{-1} (Table 4). It was found that during summer the mean was high than during winter. This might be due to the higher temperature, which increases the growth of coliform bacteria (Aleksieva and Krusher, 1981).

There were only 3 isolates of *E. coli*, which were found in the raw milk collected from Khartoum North during summer (Table 1) and the mean total count was $6\times10^5\pm7.94\times10^5$ cfu mL^{-1} (Table 2). The presence of *E. coli* in raw milk indicated fecal contamination and/or poor hygienic practices (IDF, 1994). This result is agreed with Haj Mahmoud (2002) who found the percentage of *E. coli* in raw milk to be 7.2%. Similarly Ahmed (1995) found that the average rate of isolation of *E. coli* was 15.95%. On the other hand Deutz *et al.* (1999) examined a total of 133 cow bulk milk samples for the presence of *E. coli* O157 and they found that only 1.5% of the milk samples contained *E. coli* O.157.

The rate of isolation of *Staphylococcus aureus* was found to be 46.7% in the different cities of Khartoum State (Table 1) with total mean count of $4.94\times10^4\pm1.29\times10^6$ cfu mL^{-1}. It is probable that the high incidence and high counts of *S. aureus* was favored by lack of hygiene during milking and also unavailability of cooling during transportation and storage of milk (Asperger, 1994). He also reported that nasal areas, hands and skin of human are recognized as sites of contamination. It is observed during collection of the milk samples that some employees in supermarkets practiced bad habits during selling such as snuffing and taking. Also mastitic milk could be the source of this organism

Table 3: Incidences and load of *S. aureus* and *E. coli* counts of milk samples collected from different cities in Khartoum State during summer and winter seasons

		S. aureus (cfu mL^{-1})			*E. coli* (cfu mL^{-1})		
Cities	Seasons	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
Khartoum	Winter	$1.17\times10^5\pm1.25\times10^5$	0	4.3×10^3	0	0	0
north	Summer	$2.74\times10^5\pm3.93\times10^5$	0	1.7×10^6	$6\times10^5\pm7.93\times10^5$	0	1.5×10^6
	Sub total	$2.02\times10^5\pm3.10\times10^5$	0	1.7×10^6	0	0	
Khartoum	Winter	0	0	0	0	0	0
	Summer	$3.25\times10^6\pm3.35\times10^6$	0	7.88×10^6	0	0	0
	Sub total	$3.25\times10^6\pm3.4\times10^6$	0	7.88×10^6	0	0	
Omdurman	Winter	$2.34\times10^5\pm3.39\times10^5$	0	1.1×10^6	0	0	0
	Summer	$2.93\times10^5\pm3.55\times10^5$	0	1.2×10^6	0	0	0
	Sub total	$2.63\times10^5\pm3.38\times10^5$	0	1.2×10^6	0	0	
Sub total	Winter	$1.61\times10^5\pm2.3\times10^5$	0	1.1×10^6	0	0	0
	Summer	$7.44\times10^5\pm1.66\times10^6$	0	7.88×10^6	$6\times10^5\pm 7.94\times10^5$	0	1.5×10^6
	Total	$4.94\times10^5\pm 1.29\times10^6$	2.26×10^3	0	$6\times10^5\pm 7.94\times10^5$	0	1.5×10^6

M = Mean, SD = Standard Deviation

Table 4: Comparison of microbial content of milk samples collected from different areas and cities of Khartoum State using one way ANOVA analysis

	Significant level			
Microbial measurement	Areas	Cities	Summer	Winter
TBC	0.018*	0.018*	0.03*	0.004*
S. aureus	0.001***	0.001***	0.001***	0.501^{NS}
Coliform	0.111^{NS}	0.224^{NS}	0.252^{NS}	0.531^{NS}
Spore forming bacteria	0.982^{NS}	0.528^{NS}	0.558^{NS}	0.681^{NS}

NS = Non Significant (p>0.05), * = Significant at $p\le0.05$, ** = Significant at $p\le0.01$, *** = Significant at $p\le0.001$

(Mohamed *et al*., 1997). Inadequate refrigeration and long storage before use are some factors which enhances the presence of *S. aureus* (Asperger, 1994). This high mean counts of *S. aureus* was agreed with Al-Taraz *et al.* (2003) who found *Staphylococcus* were detected in 80% of the milk samples with the count ranging from 1×10^3 to 1.3×10^6 cfu mL^{-1}. Similarly, Khan *et al.* (2002) found that mean *Staphylococcus* count was 4.7×10^8 cfu/100 mL of raw milk. Also Shamanova *et al.* (1998) found the average count of coagulase positive staphylococcus was $6.0\pm0.2\times10^2$ cfu cm^{-3}.

It was found that the highest rate of isolation was found in raw milk collected from Khartoum North (58.93%) than that of Khartoum (8.93%) and Omdurman (32.14%). Comparison of the means of the milk samples collected from the different cities and areas (Table 4) revealed significant differences ($p\le0.001$). Moreover, the milk collected from Khartoum had the highest mean count ($3.25\times10^6\pm3.4\times10^6$ cfu mL^{-1}) than those of Omdurman ($2.63\times10^5\pm3.38\times10^5$ cfu mL^{-1}) and Khartoum North ($2.02\times10^5\pm3.10\times10^5$ cfu mL^{-1}) as shown in Table 3. Higher total mean *S. aureus* count was obtained during summer ($7.44\times10^5\pm1.66\times10^6$ cfu mL^{-1}) for the milk samples collected from the three cities compared to that collected during winter ($1.61\times10^5\pm2.3\times10^5$ cfu mL^{-1}) as shown in Table 3.

Results of the present study revealed that 44.17% of the milk samples showed ring test positive reactors for Brucella (Table 1). Moreover, this indicated that nearly half of market milk is contaminated with *Brucella* spp. It was observed that the milk collected from Khartoum North had the highest percentage of Brucella (50.94%). It was also observed that any positive phosphtase test is Brucella ring test positive. This is indicated that some of the Brucella negative sample might be due to the effect of heating milk as the phosphatase enzyme was destroyed by heating. The detection of Brucella antibodies in the milk samples might be due to excretion of the antibodies by the infected or carrier cows (Ahmed, 1995). This result agreed with Haj Mahmoud (2002) who found 45.9% of the samples showed milk ring test positive reactors. Moreover, Ibrahim (1973) found that nearly all herds around Khartoum city were reactors. Suliman (1987) found 15.2% of the samples were positive for Brucella by the milk ring test. Beloti *et al.* (1999) found 4.76% of the samples were positive in the

Brucella ring test. Alves *et al.* (2001) found four positive samples and 6 suspicious samples of 79 milk samples sold in Brazil. Similarly Brucella positive isolation from milk have been reported in Turkey (Iihan *et al*., 1999), in India (Hussein *et al*., 2000), in Egypt (Abdel-Hakiem, 1999) and Oman (El-Amin *et al*., 2001).

In all samples *Salmonella* spp. were not found and this might be due to the fact that Salmonellae are rarely shed into the milk and natural infection of bovine mammary gland is possible but relatively rare (Vlaemynck, 1994). He also stated that contamination of raw milk with Salmonella usually takes place from external sources. This result is agreed with Deutz *et al*. (1999), Shamanova *et al*. (1998) and Stephan and Buhler (2002) who didn't find Salmonella in their surveys.

The present study concluded that the hygienic quality of milk is low and it supported Akasha and Al-Jibori (2003) who recommended that milking should be done under hygienic conditions and milk should be cooled immediately after milking and should be heat treated to control bacteriological quality. Hence milk must be produced, distributed, handled and marketed under the control of milk commission and the commission must have a sanitary inspector and veterinarian to enforce its methods and standards. Employees in farms and shops should be inspected at periodical intervals and they must be free from communicable diseases. Establishment and applications of Sudanese standards and grades for marketing milk and pricing structure should include the grade and the quality of milk. Further studies are needed for detection of toxins that produced by *S. aureus*, *E. coli* and spore forming bacteria (*Bacillus* spp. and *Clostridium* spp.) and other harmful microorganisms.

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