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Bacteriological Quality and Safety of Raw Cow's and Buffalo's Milk Sold in Menoufia Governorate, Egypt

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Abstract:

This study focused on assessment of the microbiological quality, including the incidence of pathogens, of commercial raw cow's and buffalo's milk in Menoufia Governorate, Egypt. A total of 70 (35 each) milk samples were collected from different sites and analyzed for microbiological quality and isolation of pathogenic bacteria. Microbiological analysis revealed that the mean aerobic plate count was $2.01\pm0.836\times10^7$ and $4.03\pm1.37\times10^7$ cfu/ml for cow's and buffalo's milk, respectively. Enterobacteriaceae were detected in 31 (88.57%) and 31 (88.57%), with mean count values of $2.22\pm0.92\times10^6$ and $1.03\pm0.267\times10^7$ cfu/ml in cow's and buffalo's milk samples, respectively. Coliforms were detected in 88.57% and 88.57%, with mean count values of $5.57\pm3.56\times10^5$ and $8.86\pm1.71\times10^5$ cfu/ml in cow's and buffalo's milk samples, respectively. *E. coli* was detected in 8 (22.9%) and 5 (14.3%), *S. aureus* was detected in 22 (62.86%) and 20 (57.14%), with mean count values of $5.69\pm1.97\times10^4$ and $1.58\pm0.50\times10^5$ cfu/ml in cow's and buffalo's milk, respectively. On the other hand, *Salmonella* and *L. monocytogenes* were not detected in the examined samples.

Keywords: *Bacteriological quality, E. coli, S. aureus, Salmonella, Listeria monocytogenes, cow's and buffalo's milk.*

Introduction

Milk is a major component in human diet all over the world, it was considered as complete food for human from birth to senility, as it contains all the nutrients required for growth and maintenance of the body health (Jay, 2000).

Milk of cattle, buffalo, goat, sheep and camel contains almost same but varying concentration of the chemical constituents. Milk differs widely in composition due to different factors including species of animal, breed, individuality, stage of lactation,

frequency of milking, age, seasonal variations, feed, interval of milking, disease and abnormal conditions and administration of drugs and hormones (Ensminger, 1993).

Cow's milk has long been considered a highly nutritious and valuable human food, and is consumed by millions daily in a variety of different products. Its nutrient composition makes it an ideal medium for bacterial growth, and therefore it can be considered one of the most perishable agricultural products because it can so very easily be contaminated (Bramley & McKinnon, 1990 and Heeschen 1994).

Buffalo's milk receives increasing research interest and investment in various countries, owing mainly to its attractive nutrient content (Amarjit & Toshihiko, 2003). Buffalo is the second most important dairy species in the world. Egypt is among the largest producer countries of buffalo milk, with both buffalo herds and buffalo milk production listed forth worldwide in 2008, after those of India, Pakistan and China (FAOSTAT, 2008).

Raw milk could be a source of undesirable or even pathogenic bacteria which implicated in milkborne diseases. A number of bacteria including *S. aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* have been recovered from raw milk and some of these have been determined to be pathogenic and toxicogenic, and implicated in milkborne gastroenteritis (De Buyser et al., 2001; Harrington et al., 2002)

Microorganisms may gain entry into raw cow's and buffalo's milk from various sources either directly from dairy animals experiencing sub clinical or clinical mastitis, or from faecal contamination, particularly around the teats, and from the farm environment particularly the water source and utensils used for the storage of milk on farm or during transportation (Oliver et al., 2005).

In view of the growing public awareness about food safety and quality, a better knowledge of the microbiological quality of milk is of great significance for further development of its hygienic processing to safeguard the consumers. Therefore the objectives of this study were to 1) determine the microbiological status of cow's and buffalo's milk sold in supermarkets in Menoufia governorate, Egypt and 2) study the prevalence of foodborne pathogens, especially *E. coli*, *S. aureus*, *Salmonella* spp. and *L. monocytogenes* in cow's and buffalo's milk.

Materials and Methods

Collection of samples:

Seventy raw cow's and buffalo's milk samples (35 each) were collected from dairy shops and supermarkets from different areas in Menoufia Governorate. Collected samples were transferred to the laboratory of Food Hygiene

& Control Department at University of Sadat city in an ice box for bacteriological examination.

Bacteriological examination:

Initially, 25 ml of each raw milk sample was dispensed into a sterile flask containing 225 ml of 0.1% peptone water and mixed thoroughly. Subsequent serial decimal dilutions of each sample were prepared in 0.1% peptone water.

Viable cell counts were performed by the standard pour plate method after serial dilutions in the following conditions: Aerobic plate count (APC) was carried out on plate count agar according to the plate count method APHA 2001 (Morton, 2001).

Enterobacteriaceae count was carried out on Violet Red Bile Glucose (VRBG) Agar according to the plate count method APHA 2001 (Kornacki & Johnson, 2001).

Coliform bacteria were enumerated by the most probable number (MPN) multiple-tube fermentation method according to US standard method (US FDA, 2002). The identification of *E. coli* was confirmed by colony morphology on eosin methylene blue agar (EMB) and performing biochemical tests according to Holt et al. (1994). Serological identification of isolated *E. coli* was done according to Varnam & Evans (1991).

S. aureus count was carried out by direct plate count method on Baird Parker agar supplemented with egg yolk tellurite emulsion according to the plate count method APHA 2001 (Lancette & Bennett, 2001).

Detection of Salmonella:

Detection of Salmonella was done using the presence/absence method (US FDA, 2011). The suspected isolates were identified according to Forbes et al. (2007).

Detection of Listeria monocytogenes:

Detection of *L. monocytogenes* was done according to the most widely used approaches which based upon FDA method (Lovett, 1987) modified by Hitchins (1990). Identification of suspected colonies was done according to Hitchins (1995)

Results and Discussion

The analyzed samples were in general highly contaminated with the tested bacterial groups (Table 1). The aerobic plate count (APC) is an indication of the sanitary conditions under which the food was produced (Andrews, 1992). The results obtained in this study showed that all examined samples of raw cow's and buffalo's milk were contaminated with aerobic mesophilic bacteria, and the APC/ml ranged from 8.8×10^4 to 2.78×10^8 and 4.9×10^4 to 4.3×10^8 with mean count values of $2.01\pm0.83\times10^6$ and $4.03\pm1.37\times10^7$ respectively (Table 1). The highest frequency distribution of APC in examined raw cow's

and buffalo's milk samples were 74.28% and 68.57%, lies within the range of 10^6 to $<10^8$ and 10^6 to $<10^8$ respectively (Figures 1&2).

These findings for raw cow's milk, agree to some extent with those reported by Godefay and Molla (2000), Chye et al. (2004), Mennane et al. (2007) and Abd El-Krim et al. (2008), while relatively lower counts were reported by Kivaria et al. (2006), El-Diasty & El- Kaseh (2007), while comparatively higher counts were recorded by Tarek (2000) and Sobeih et al. (2002). The obtained findings for raw buffalo's milk were approached those reported by Adesiyun (1994), Awadall (2002) and Muhammad et al. (2009), relatively lower counts were reported by Boycheva et al (2002), Chatterjee et al. (2006) and Han et al. (2007). Comparatively higher counts were recorded by Tarek (2000) and Ibrahim (2010).

According to the limits proposed by Egyptian Standards (ES, 2010), recommended by the Egyptian Organization for Standardization and Quality "EOSQ", SPC of raw milk must not exceed 1×10^5 cfu/ml milk. Only 2.86% and 8.57% of the examined raw cow's and buffalo's milk samples complied with the standard, respectively.

The same percentage (88.6%) of the examined raw cow's and buffalo's milk samples were contaminated with Enterobacteriaceae with counts ranged from 9.0×10^2 to 2.5×10^7 and 3.5×10^3 to 5.310^7 with mean count values of $2.22 \pm 0.92\times10^6$ and $1.03\pm0.27\times10^7$ respectively (Table 1). The highest frequency distribution of Enterobacteriaceae count of the examined raw cow's and buffalo's milk samples were 77.14% and 62.85%, lies within the range of 10^4 to $<10^7$ and 10^6 to $<10^8$ (Figures 1&2)

The obtained findings for raw cow's milk are concomitant with those reported by Allam (1999) and El-Diasty & El-Kaseh (2007). Comparatively higher findings were recorded by El-Zubeir & Ahmed (2007). For raw buffalo's milk, comparatively higher findings were recorded by El-Shazly (2007). The obtained higher incidences and counts may be attributed to the unhygienic condition under which milk was produced, handled and stored, and is an indicative for direct or indirect feacal pollution of milk, neglection of hygienic measures and possible presence of enteric pathogens (Jay, 2000). The incidence of coliforms were detected at the same percentage (88.6%) and the counts ranged from 2×10^2 to 1.1×10^7 and 9×10^2 to 5×10^6 with mean count values of $5.57\pm3.56\times10^5$ and $8.86\pm1.71\times10^5$ for examined raw cow's and buffalo's milk samples respectively (Table 1).

Table 1. Bacterial loads of commercial raw cow's and buffalo's milk

Bacterial counts	Cow milk (n=35)					Buffalo milk (n=35)				
	Positive samples		Min.	Max.	Mean ±SEM	Positive samples		Min.	Max.	Mean ±SEM
	No.	%				No.	%			
APC	35	100	8.8×10^4	2.78×10^8	$2.01 \pm 0.84 \times 10^7$	35	100	4.9×10^4	4.3×10^8	$4.03 \pm 1.37 \times 10^7$
Enterobacteriaceae	31	88.6	9×10^2	2.5×10^7	$2.22 \pm 0.92 \times 10^6$	31	88.6	3.5×10^3	5.3×10^7	$1.03 \pm 0.267 \times 10^7$
Coliforms	31	88.6	2×10^2	1.1×10^7	$5.57 \pm 3.56 \times 10^5$	31	88.6	9×10^2	5×10^6	$8.86 \pm 1.71 \times 10^5$
S. aureus	22	62.9	1.7×10^3	3.4×10^5	$5.69 \pm 1.97 \times 10^4$	20	57.1	1×10^3	6×10^5	$1.58 \pm 0.50 \times 10^5$

No. of examined samples = 35

*SEM= Standard error of the mean

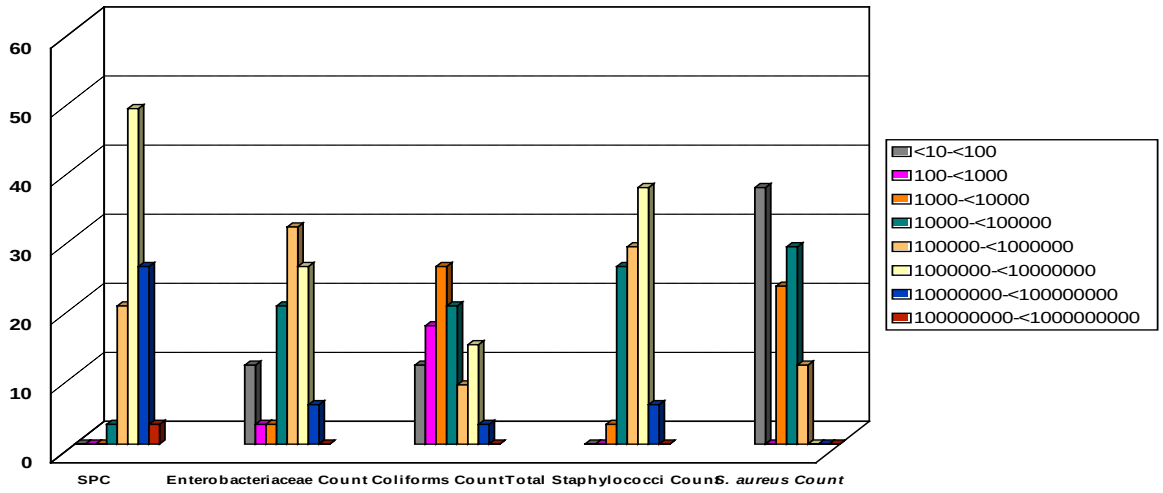

Fig. (1) Frequency distribution of bacterial load in cow's milk samples

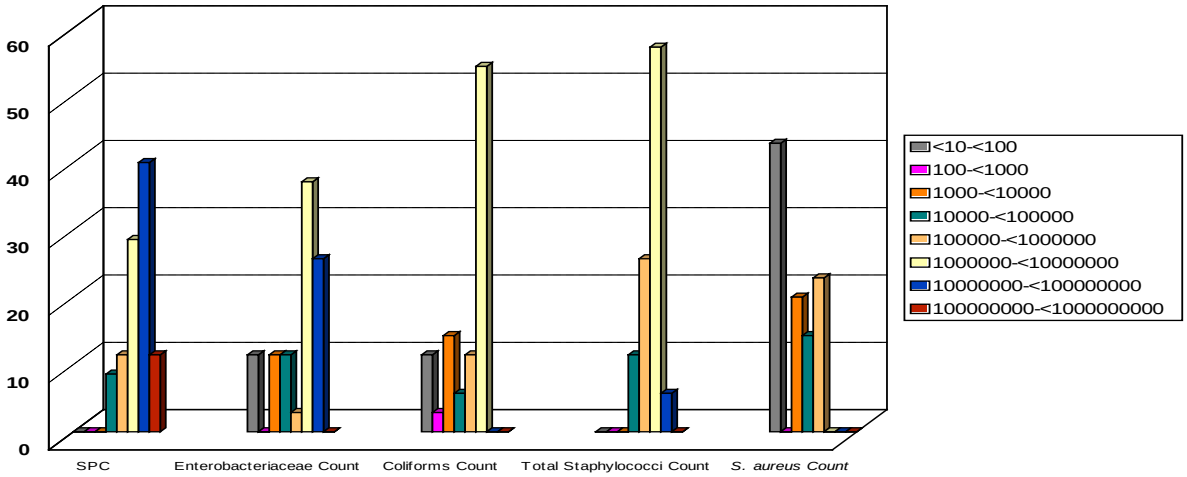

Fig. (2) Frequency distribution of bacterial load in buffalo's milk samples

The highest frequency distribution of coliforms in raw cow's and buffalo's milk samples were 62.85% and 65.72%, lies within the range of 10^2 to $<10^5$ and 10^5 to $<10^7$, respectively (Figures 1&2). Nearly similar findings for raw cow's milk were recorded by Al-Tarazi et al., (2003) and Abd El-Krim et al. (2008). Comparatively higher counts were recorded by Saudi & Mowad (1990) and El-Diasty & El-Kaseh (2007) and relatively lower counts were recorded Tarek (2000) and Mennane et al. (2007). The obtained findings for raw buffalo's milk agree to some extent to that obtained by Tarek (2000) and Awadall (2002). Relatively higher counts were reported by Hafez (1984), Farag (1987), and El-Shazly (2007), while relatively lower counts were obtained by El-Sayed & Ayoub (1993) and Wira & Orasa (2009).

Coliforms are abundant in the environment which including dust, manure, hair coat, exterior of the udder and milkers hand. Moreover, coliforms in milk can reduce its keeping quality due to production of sharp flavored substances (Blood et al., 1983). Furthermore, presence of coliforms and faecal coliforms beyond certain level could be of public health hazard, as they may cause dreadful diarrheal diseases (Robert et al., 1977).

The predominant isolated coliform strains in the examined raw cow's and buffalo's milk samples were. *E. coli, Citrobacter amalonaticus, C. freundii, Escherichia adecarboxylata, E. coli inactive, Enterobacter aerogenes, Ent. agglomerans, Ent. cloacae, Ent. gergoviae, Klebsiella oxytoca, K. pnumoniae sub spp. ozaenae, and K. pneumoniae sub.spp. pneumoniae* at percentages of (22.86& 14.29%), (14.29& 8.57%), (0& 5.71%), (2.86& 0%), (8.57%& 8.57%), (11.43& 5.71 %), (28.57& 14.29%), (0& 5.71%), (17.14& 11.43%) (11.43& 14.29%), (11.43& 20%) and (8.57& 20%), respectively (Table 2). Serological typing of isolated *E. coli* showed that they belonged to EPEC serotypes O119, O55 and O127:K63 and EIEC O124:K72 while the remaining were untypable (Table 3). The presence of presumably pathogenic *S. aureus* in 62.86% and 57.14% of examined raw cow's and buffalo's milk with counts ranged from 1.7×10^3 to 3.4×10^5 and 1.0×10^3 to 6.0×10^5 with mean count values of $5.69\pm1.97\times10^4$ and $1.58\pm0.50\times10^5$ respectively (Table 1), indicates the poor hygienic quality under which such milk was produced and also may indicate udder inflammation as staphylococcus spp. are one of the main etiological agents of intramammary infections.

Table 2. Incidence of Coliform organisms in the examined cow's and buffalo's milk samples

Isolates	Cow milk (n=35)		Buffalo milk (n=35)	
	Positive samples		Positive samples	
	No.	%.*	No.	%*
Escherichia coli	8	22.9#	5	14.3
Citrobacter amalonaticus	5	14.3	3	8.57
C.freundii	0	0	2	5.71
Escherichia adecarboxylata	1	2.86	0	0
E.coli inactive	3	8.57	3	8.57
Enterobacter aerogenes	4	11.4	2	5.71
Ent. agglomerans	10	28.6	5	14.3
Ent. cloacae	0	0	2	5.71
Ent. gergoviae	6	17.14	4	11.4
Klebsiella oxytoca	4	11.43	5	14.3
K. pneumoniae sub.spp. ozaenae	4	11.43	7	20
K. pneumoniae sub.spp. pneumoniae	3	8.57	7	20

* **% calculated according to samples number**.

Table 3. Serodiagnosis of some *E. coli* strains isolated from the examined cow's and buffalo's milk samples.

E. coli serotype	Cow milk (n=35)	Buffalo milk (n=35)	Strain pathotype
	No. of strains (%) #	No. of strains (%)	
O_{55}:K_{59}	-	1 (2.9)	EPEC
O_{119}:K_{69}	1 (2.9)	-	EPEC
O_{124}:K_{72}	1 (2.9)	2 (5.7)	EIEC
O_{127}:K_{63}	1 (2.9)	-	EPEC
Untypable	2 (5.7)	1 (2.9)	-
Total	5 (14.3)	4 (11.4)	

EPEC =Enteropathogenic *E.coli*
# % calculated according to samples number.

Table 4. Incidence of *Salmonella* spp. and *L. monocytogenes* in the examined cow's and buffalo's samples

Isolated bacteria	Cow milk (n = 35)		Ewe's milk (n = 35)	
	No.	%	No.	%
Salmonella spp.	0	0	0	0
L. monocytogenes	0	0	0	0

The highest frequency distribution of *S. aureus* in examined raw cow's and buffalo's milk samples were 51.43% and 57.15%, lies within the range of 10^3 to $<10^5$ and 10^3 to $<10^6$ respectively (Figures 1&2).

Nearly similar findings for raw cow's milk were obtained by Desmasures et al. (1997), Ali (2000) and Mohamed et al. (2002),

relatively higher counts and incidence were reported by Halawa (1987), Capurro et al. (2000) and Mennane et al. (2007),. Relatively lower counts and incidence were obtained by El-Bagoury (1992), and Belickova et al. (2000). For raw buffalo's milk, nearly similar findings were obtained by Adesiyum (1994) and Awadall (2002), relatively higher counts and incidence were reported by Halawa (1987), El-Bagoury (1988) and Jorgensen et al. (2005). Relatively lower counts and incidence were obtained by Gupta (1986) and Youssef et al. (2010).

Comparing the obtained results with Egyptian Standard (ES, 2005) recommended by the Egyptian Organization for Standardization and Quality "EOSQ", which stipulated that the number of *S. aureus* must not exceed 100 cfu/ ml, only 37.14% and 42.86% of examined raw cow's and buffalo's milk samples, respectively, complied with the standard.

Salmonella and *L. monocytogenes* were not detected in any of examined samples (Table 4). These findings, agree with results recorded by Nero et al. (2008), D'Amico & Donnelly (2010). Raw milk must be *Salmonella* and *L. monocytogenes* free (ES, 2005). Consequently all examined raw cow's and buffalo's samples complied with the standard in this point.

Conclusion

Results obtained in this study highlight the poor microbiological and sanitary quality of raw cow's and buffalo's milk sold in supermarkets in Menoufia governorate, and showed that the prevalence and counts of Enterobacteriaceae, coliforms and *S. aureus* were higher compared to some other studies. Therefore more efforts should be taken to increase sanitary and hygienic measures during production, transportation and storage of cow's and buffalo's milk to safe guard the consumers.

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