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Bacteriological quality and occurrence of some microbial pathogens in goat's and ewe's milk in Egypt

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Abstract

This study investigated the microbiological quality and the incidence of some pathogens in raw goat's and ewe's milk in Egypt. Microbiological analysis revealed that the mean aerobic plate count was $9.11\pm2.47\times10^6$ and $2.04\pm0.91\times10^6$ CFU/ml for goat's and ewe's milk, respectively. Enterobacteriaceae were detected in 24 (68.6%) goat's milk samples and 21 (60%) ewe's milk samples, with mean count values of $2.53\pm0.57\times10^6$ and $1.67\pm0.87\times10^5$ CFU/ml in goat's and ewe's milk samples, respectively. Coliforms were detected in 68.57% and 60%, with mean count values of $6.47\pm2.17\times10^5$ and $1.66\pm0.85\times10^5$ CFU/ml in goat's and ewe's milk samples, respectively. *Escherichia coli* was detected in 5 (14.3%) and 4 (11.4%), *Staphylococcus aureus* was detected in 11 (31.43%) and 13 (37.14%), with mean count values of 1.41×10^4 and 6.67×10^4 CFU/ml in goat's and ewe's milk, respectively. On the other hand, *Salmonella* and *Listeria monocytogenes* were not detected in the examined samples. Obtained results highlighted the poor microbiological and sanitary quality of goat's and ewe's milk produced in Egypt.

Introduction

In Mediterranean countries, goat's and ewe's milk are gaining considerably in economic importance as a result of growing acceptance of products made from them, especially cheeses (Miguel *et al*., 1997). Unlike cow's milk, which has rigorous hygiene and quality regulations, microbiological standards for the production and distribution of goat and sheep milk are less strict, although there are increasing demands for their milk by consumers (Haenlein, 2004; Zweifel *et al*., 2005).

Public health hazards associated with consumption of raw cow's milk and its products have been well documented (De Buyser *et al*., 2001; Harrington *et al.*, 2002). There is no evidence that the health hazards from raw goat's or ewe's milk is any lower (Mcintyre *et al*., 2002). Goat's and sheep's milk can be, similar to cow's milk, source of undesirable or even pathogenic bacteria which implicated in milkborne diseases including *Listeria monocytogenes, Escherichia coli, Salmonella* spp. and *Staphylococcus aureus* (Gaya *et al*., 1996; Rampling, 1998; Foschino *et al.*, 2002; Muehlherr *et al*., 2003). These microorganisms could gain access to milk either from faecal contamination, particularly around the teats, or by direct excretion from the udder.

Little data is available on the microbiological quality and safety of ovine milk in Egypt. Given the increased demand for ovine milk and their products worldwide, this issue has recently received more attention. A better knowledge of the microbiological quality of raw goat's and ewe's milk will contribute to further research aimed at the improvement of their quality and in turn improvement of cheese made from them as the cheese quality depends closely on the quality of milk. Therefore, the objectives of this study were to determine the microbiological status of goat's and ewe's milk in Egypt as well as to study the prevalence and counts of some foodborne pathogens, especially *E. coli, S. aureus, Salmonella* spp. and *L. monocytogenes* in goat's and ewe's milk.

Materials and Methods

Collection of samples

Seventy raw ewe's and goat's milk samples (35, each) were collected from ewe and goat flocks at Menoufia Governorate. Samples were collected aseptically in clean, dry and sterile sampling bags which placed in an insulated sampling case containing ice to ensure a storage temperature around 4°C and transported to the laboratory of Food Hygiene and Control Department at University of Sadat city for bacteriological examination.

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Bacteriological examination

Initially, 25 ml of each raw milk sample 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) carried out on plate count agar according to the plate count method APHA 2001 (Morton, 2001). Enterobacteriaceae count carried out on Violet Red Bile Glucose (VRBG) Agar according to the plate count method APHA 2001 (Kornacki and 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* and other coliform bacteria were 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 and Evans (1991). *Staphylococcus aureus* count 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 and 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 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. 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 goat's and ewe's milk were contaminated with aerobic mesophilic bacteria with high mean count values (Table 1). Similar findings from other countries were reported (Morgan *et al.*, 2003; Muehlherr *et al.*, 2003). Relatively lower counts were reported by Stubbs and Hacking (1986) (65% of samples with count $>2.0\times10^4$ CFU/ml), Zeng and Escobar (1996) (9×10^2 CFU/ml) and Zweifel *et al*. (2005) (3.98×10^4 CFU/ml), while comparatively higher counts were recorded by Perez-Elortondo *et al.* (1990) (10^8 to 10^{12} CFU/ml), Vujicic and Vulic (1992) (1.7×10^7 CFU/ml) and Salem (2003) (10^7 to 10^9 CFU/ml).

68.57 and 60% of examined raw goat's and ewe's milk samples were contaminated with Enterobacteriaceae, with mean count values of $2.53\pm0.57\times10^6$ and $1.67\pm0.87\times10^5$ CFU/ml, respectively (Table 1). Comparatively lower findings were recorded by Muehlherr *et al.* (2003), for raw goat's milk (61.5%), while the findings recorded by the same authors are higher than that obtained in this study for raw ewe's milk (71.4%). On the other hand relatively lower counts (4.4×10^2 to 6.2×10^3 CFU/ml) were recorded by Gaya *et al.* (1987).

The incidences of coliforms in the examined samples was 68.57% and 60%, for raw goat's and ewe's milk, respectively (Table 1). Comparatively lower counts were recorded by Roberts (1985), Nazem and Thanaa (1993) and Salem (2003) for raw goat's milk, and relatively lower counts were recorded by Gaya *et al.* (1987) and Bahout (1995) for raw ewe's milk. Coliform counts as thousands CFU/ml may indicate a problem of dirty goats or ewe being milked; an unclean udder, unsanitary milking practices, or milk contamination in the container (Wasiksiri *et al*., 2010).

The predominant isolated coliform strains in the examined raw goat's and ewe's milk samples were. *E. coli, Citrobacter amalonaticus, C. freundii, Escherichia adecarboxylata, Enterobacter aerogenes, Ent. agglomerans, Ent. cloacae, Ent. gergoviae, Klebsiella oxytoca, K. pnumoniae sub spp. ozaenae, K. pneumoniae* sub.spp. *pneumoniae* and *Hafnia alvei* at percentages varied between 0 to 17.14% (Table 2). Types of isolated coliforms could be isolated with different percentages by Gaya *et al*. (1987), Erer *et al.* (1990), Garcia-Armesto *et al.* (1993) and Bahout (1995).

The incidence of *E. coli* (14.29 and11.43% for raw goat's and ewe's milk, respectively) in this study agrees to some extent with that recorded by Ates *et al.* (1990) and Giannotti *et al.* (1993). Relatively lower incidence was reported by Little and Louvois (1999), Foschino *et al.* (2002) and Dontorou *et al*. (2003), while relatively higher incidence was reported by Jensen and Hughes (1980), Roberts (1985), Gaya *et al.* (1987), Bahout (1995) and Abd El-Aal and Awad (2008). Serological typing of isolated *E. coli* showed

Table 1. Mean counts (CFU/ml) and occurrence of some bacterial pathogens with a hygienic significance in goat's and ewe's milk

Bacterial counts	Goat's milk (n=35)					Ewe's milk (n=35)				
	Positive samples		Min.	Max.	Mean ±SEM	Positive samples		Min.	Max.	Mean ±SEM
	No.	%				No.	%			
APC	35	100	1×10^4	6×10^7	$9.11 \pm 2.47 \times 10^6$	35	100	1×10^4	2.88×10^7	$2.04 \pm 0.915 \times 10^6$
Enterobacteriaceae	24	68.57	2.3×10^3	8.5×10^6	$2.53 \pm 0.569 \times 10^6$	21	60	30	1.5×10^6	$1.67 \pm 0.873 \times 10^5$
Coliforms	24	68.57	2.1×10^2	5×10^6	$6.47 \pm 2.17 \times 10^5$	21	60	7	1.1×10^6	$1.66 \pm 0.854 \times 10^5$
S. aureus	11	31.43	1×10^3	3×10^5	$6.67 \pm 2.71 \times 10^4$	13	37.14	1×10^2	7×10^4	$1.41 \pm 0.574 \times 10^4$

No. of examined samples = 35 *SEM= Standard error of the mean

Table 2. Occurrence of Coliform organisms in the examined goat's and ewe's milk samples

Isolates	Goat's milk (n=35)		Ewe's milk (n=35)	
	Positive samples		Positive samples	
	No.	%.[a]	No.	%[a]
E. coli	5	14.3	4	11.4
Citrobacter amalonaticus	3	8.6	0	0
C. freundii	2	5.7	0	0
Escherichia adecarboxylata	2	5.7	0	0
Enterobacter aerogenes	2	5.7	1	2.9
Ent. agglomerans	3	8.6	2	5.7
Ent. cloacae	4	11.4	3	8.6
Ent. gergoviae	0	0	1	2.9
Klebsiella oxytoca	4	11.4	0	0
*K. pneumoniae sub.*spp. *ozaenae*	2	5.7	5	14.3
K. pneumoniae sub.spp. pneumoniae	6	17.1	0	0
Hafnia alvei	5	14.3	6	17.1

[a] % calculated according to samples number.

Table 3. Serotyping of *E. coli* strains isolated from the examined goat's t and ewe's milk.

E. coli serotype	Goat's milk (n=35)	Ewe's milk (n=35)	Strain pathotype
	No. of strains (%)[a]	No. of strains (%)	
$O_{55}:K_{59}$	1 (2.9)	1 (2.9)	EPEC
$O_{119}:K_{69}$	-	1 (2.9)	EPEC
$O_{158}:K-$	-	1 (2.9)	EPEC
Untypable	4 (5.7)	1 (2.9)	-
Total	5 (14.3)	4 (11.4)	

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

that they belonged to EPEC serotypes O119, O55 and O158 while the remaining were untypable (Table 3). Sheep and goats may act as a reservoir of pathogenic *E. coli* and their milk may serve as vehicle for the pathogen transmission to humans (Abd El-Aal and Awad, 2008).

The presence of presumably pathogenic *S. aureus* in 31.43 and 37.14% of examined raw goat's and ewe's milk with with mean count values of $1.41 \pm 0.57 \times 10^4$ and $6.67 \pm 2.71 \times 10^4$, respectively, indicates the poor hygienic quality under which such milk was produced and also may indicate udder inflammation as staphylococcus spp. are the main etiological agents of small ruminant's intramammary infections, and the more frequent isolates being *S. aureus* (Bergonier *et al*., 2003). Nearly similar findings were obtained by Erer *et al*. (1990), Foschino *et al.* (2002) and Muehlherr *et al.* (2003), relatively higher incidence was reported by Kornel (1992) and Jakobsen *et al.* (2011), while relatively lower counts and incidence were obtained by Little and Louvois (1999), Muehlherr *et al.* (2003) and Salem (2003).

The high figures of contamination with the tested bacterial groups may be due to the rearing system of small ruminants, which still primitive in many countries and make it difficult both to minimize environmental bacterial contamination at the milking stage, and to carry out effective milk quality improvement programs. On the other hand the low counts obtained by some authors may reflect the good sanitation practices applied in the farm and during milking process.

Salmonella and *L. monocytogenes* were not detected in any of the examined samples. The absence of these two dangerous microorganisms in raw ovine milk has also been pointed out by others (Little and Louvois, 1999; Vashin *et al*. 1999; Foschino *et al*., 2002; Muehlherr *et al*., 2003; Ekici *et al.*, 2004; Abd El-Aal and Awad, 2008; Jakobsen *et al.*, 2011).

There are a series of factors behind the difficulties in managing the sanitary quality, and play a role in contamination, of sheep and goat milk with various microorganisms. These factors include the low level of production per head, relatively small flocks, poor milking facilities, poor water supply, dirty teats and udder, because are nearer to the soil, the small ruminants and particularly the ewe is a very hairy animal which contributes to the bacterial contamination of the milk, hand-milking and consequently, long milking times, the conditions under which the herds or flocks are raised, adverse climatic conditions and the spread of production over a wide geographic area (Kalantzopoulos *et al*., 2002).

Conclusion

Results obtained in this study highlighted the

poor microbiological and sanitary quality of goat and sheep milk produced in Egypt, and showed that the prevalence and counts of Enterobacteriaceae, coliforms and *S. aureus* were higher compared to some other studies from other countries. Therefore, more efforts should be taken to increase sanitary and hygienic measures during production of goat and sheep milk to safe guard the consumers.

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