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±2.47×10^6 and 2.04±0.91×10^6 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±0.57×10^6 and 1.67±0.87×10^5 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±2.17×10^5 and 1.66±0.85×10^5 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.

Keywords
Bacteriological quality
Escherichia coli
Staphylococcus aureus
Goat’s and Ewe’s milk

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 Menoufa 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.
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 >2.0×10⁶ CFU/ml), Zeng and Escobar (1996) (9×10² CFU/ml) and Zweifel et al. (2005) (3.98×10⁷ CFU/ml), while comparatively higher counts were recorded by Perez-Elortondo et al. (1990) (10⁶ to 10¹⁰ CFU/ml), Vujicic and Vulic (1992) (1.7×10⁷ CFU/ml) and Salem (2003) (10¹ to 10⁶ 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±0.57×10⁶ and 1.67±0.87×10⁶ 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 (4.4×10⁶ to 6.2×10⁶ CFU/ml) were recorded by Gay 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 Gay 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 aderacboxylata, Enterobacter aerogenes, Ent. agglomerans, Ent. cloacae, Ent. gergoviae, Klebsiella oxytoca, K. pneumoniae 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 Gay 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 recorded by Little and Louvois (1999), Foschinio et al. (2002) and Dontorou et al. (2003), while relatively higher incidence was reported by Jensen and Hughes (1980), Roberts (1985), Gay et al. (1987), Bahout (1995) and Abd El-Aal and Awad (2008). Serological typing of isolated E. coli showed
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 mean count values of 1.41±0.57×10^4 and 6.67±2.71×10^4, 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

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

<table>
<thead>
<tr>
<th>Bacterial counts</th>
<th>Goat’s milk (n=35)</th>
<th>Ewe’s milk (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive samples</td>
<td>Min.</td>
</tr>
<tr>
<td><strong>APC</strong></td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>24</td>
<td>68.57</td>
</tr>
<tr>
<td><strong>Coliforms</strong></td>
<td>24</td>
<td>68.57</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>11</td>
<td>31.43</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Goat’s milk (n=35)</th>
<th>Ewe’s milk (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive samples</td>
<td>No.</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Citrobacter amylolentus</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>C. freundi</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Escherichia adolestoxia</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Ent. amylovarum</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Ent. cloacae</td>
<td>4</td>
<td>11.4</td>
</tr>
<tr>
<td>Ent. gengoeae</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>4</td>
<td>11.4</td>
</tr>
<tr>
<td>K. pneumoniae sub spp. oxygenae</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>K. pneumoniae sub spp. pneumoniae</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>Hafnia alvei</td>
<td>5</td>
<td>14.3</td>
</tr>
</tbody>
</table>

*SEM* calculated according to samples number.

**Table 3. Serotyping of *E. coli* strains isolated from the examined goat’s and ewe’s milk.**

<table>
<thead>
<tr>
<th><em>E. coli</em> serotype</th>
<th>Goat’s milk (n=35)</th>
<th>Ewe’s milk (n=35)</th>
<th>Strain pathotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of strains (%)</td>
<td>No. of strains (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O157:K6</td>
<td>1 (29)</td>
<td>1 (29)</td>
<td>EPEC</td>
</tr>
<tr>
<td>O11:K6</td>
<td>-</td>
<td>1 (29)</td>
<td>EPEC</td>
</tr>
<tr>
<td>O155:K-</td>
<td>-</td>
<td>1 (29)</td>
<td>EPEC</td>
</tr>
<tr>
<td>Untypable</td>
<td>4 (5.7)</td>
<td>1 (29)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>5 (14.3)</td>
<td>4 (11.4)</td>
<td></td>
</tr>
</tbody>
</table>

EPEC = Enteropathogenic *E. coli*.

*SEM* calculated according to samples number.
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.

References


Little, C. L. and Louvois, J. 1999. Health risks associated


