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Bacteriological quality of raw ewe's and goat's milk, with special references to foodborne pathogens

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Fifty raw ewe's and goat's milk samples (25 of each) were examined for total viable, psychrotrophic count and the presence of foodborne pathogenic microorganisms. The obtained results revealed that the mean total bacterial counts/ml were 1.9 x10^3 and 1.4 x10^3 in the examined samples, respectively. Psychrotrophic bacteria could be detected in all examined samples (100.0 %) with mean values of 7.8 x10 and 6.3 x10/mL, respectively. *Staphylococci, Enterococci,* and *E. coli*, were detected in (52.0 & 84.0 %), (44.0 & 36.0 %) and (36.0 & 44.0 %) of the examined samples with mean values/ml of (7.2 x10 & 6.1 x10), (2.5 x10 & 2.4 x10) and (3.0 x10 & 2.1x10), respectively. The predominant isolated bacterial strains were *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Enterococcus faecium* and *E. coli*, at percentages of (24.3 & 19.2 %), (16.2 & 32.7 %), (10.8 & 13.5 %), (19.0 & 17.3 %) and (29.7 & 17.3 %) of total isolates, respectively. On the other hand, *Clostridium perfringens, Campylobacter jejuni, Corynebacterium bovis* and *Salmonellae* failed to be detected in all examined samples. The sanitary and public health importance of these organisms as well as preventive measures to improve the quality of milk and safeguard the consumers from infection were discussed.

Although the world production of goat's milk has been relatively minor when compared with total of bovine milk (2.1 % versus 84.6 % of the total production, respectively), the worldwide goat population has reached 758 million heads with 55 % increase during the last 20 years, and 12.2 million tons of goat's milk with 58 % increasing during the same period (FAO, 2004). Also, more than two million metric tons of ewe's milk are produced in European Union (Herrero, 1999).

There are growing demands for ovine milk by consumers. This is due to the increasing number of children suffering from intolerance to cow's milk (Zweifel *et al.,* 2005). Ewe's and goat's milk are currently gaining considerably in economic importance, particularly in Mediterranean countries, as a result of growing acceptance of products made from them, mainly cheeses (Miguel *et al.,* 1997).In developing countries, the production of these types of milk are coming to be useful strategy to tackle the problem of under nutrition, especially among the infant population (Haenlein, 2004).

In most regions ovine milk is used for production of cheese and salted yoghurt, which is one of the most liked traditional dairy products because it is smoother and whiter as compared with cow's milk product, higher digestibility, distinct alkalinity, higher buffering capacity, and certain therapeutic value in medicine and human nutrition (Haenlein, 2004).

The microbiological characteristics of ovine milk differ from bovine milk in certain respects. Such factors as the larger number of head per volume of milk production (low level of production per head), the large number of head per flock, feeding, the milking process (the difficulty involved in machine milking), the conditions under which the herds or flocks are raised, adverse climatic conditions and the spread of production over a wide geographic area ect., all increase the difficulty of establishing good sanitary practices during milk production (Salmeron *et al.,* 2002). According to compositional differences between the milk from cows, goats and ewes, quality standards adjusted and evaluated for specifics of small ruminant's milk should be considered (Morgan *et al.,* 2000). The microbiological standard set by E C Directive 71/96 indicates a maximum permissible total count of 5 x10^{5}cfu/ml for raw ovine milk intended for direct use in manufacturing of dairy products (DOCE, 1994).

Raw ovine milk has been implicated as an important source of infection with the common bacterial agents associated with gastroenteritis as Salmonella spp, Staphylococci, Enterococci, and

E. coli (Tamblyn, 1986). In view of food safety and consumer health protection, however, evaluation of microbiological status of ewe's and goat's milk and to evaluate whether possible foodborne pathogens are associated with these types of milk.

Materials and methods

Collection of samples. Fifty raw ewe's and goat's milk samples (25 of each) were collected from the Lindenhof farm of Hohenheim University. The samples were collected aseptically (about 50 ml each) in clean, dry and sterile sampling bottles which placed in an insulated sampling case containing ice to ensure a storage temperature 4°C and transported to the laboratory of Umwelt und Teirhygiene Institute for bacteriological examination.

Preparation of samples. Each sample of milk was thoroughly mixed before being subjected to bacteriological examination. One ml of each prepared sample was added to 9 ml of sterile saline to make serial decimal dilutions (A.P.H.A., 1992).

Bacteriological examination.

Total colony count (A.P.H.A., 1992). 0.1 ml from the previously prepared dilution was inoculated onto duplicates of standard plate count (SPC) agar and incubated at 37°C for 24-48 hrs.

Enumeration of psychrotrophic microorganisms (A.P.H.A., 1992). From each previously prepared serial dilution of the samples 0.1 ml was inoculated onto duplicates of standard plate count agar medium. Both inoculated and control plates were incubated at 7°C for 10 days. Total psychrotrophic count/ml of examined samples were calculated and recorded.

Staphylococci count (Chapman, 1945). 0.1 ml from the previously prepared dilutions of the examined samples was transferred and evenly spread on the surface of Mannitol salt agar medium (Oxoid, 1990) plates. Inoculated plates were incubated at 37°C for 48 h. and Staphylococci count/ml was calculated and recorded.

Enterococci count (Gelsomino *et al.,* 2003). 0.1 ml from the previously prepared dilutions of the examined samples was inoculated on the surface of kanamycin esculin azide agar (kAA; Merck, Darmstadt, Germany). Inoculated plates were incubated at 37°C. Counts were determined after 24 h incubation. black and gray colonies were counted. Total Enterococci counts/ml of examined samples were calculated and recorded.

***E. coli* count.** 0.1 ml from the previously prepared dilutions of the examined samples was spread onto Targitol medium and Endo medium (Oxoid, 1990) which incubated at 37°C for 24 hrs.

Isolation of Salmonella spp. (Jayarao and Henning, 2001).

Pre-enrichment. 25 ml milk sample were added to 225 ml peptone water with Novobiocin (Standard: 100 mg/1 ml sterile D.W. & Test: 0.9 ml prepared solution/225) then incubated at 37°C for 24 hrs.

Selective enrichment. 1 ml peptone water from previously prepared pre–enrichment was added to 10 ml Rappaport Vassiliadis broth, Difco Laboratories (Two tubes) after that one tube was incubated at 37°C for 24 h. and the second at 43°C for 24 hrs.

Plating on selective medium. 0.1 ml of incubated Rappaport evenly spread on the surface of Xylose Lysine Desoxycholate (XLD) and Brillient Green Phenol Red Lactose Sucrose (BPLS) plates, Unipath Co**.** Inoculated plates were incubated at 37°C for 24 h and 43°C for 24 hrs.

Anaerobic spore formers count. Using Thioglucolate medium (Oxoid, 1990). 1 ml milk sample was inoculated in 3 tubes of Thioglucolate broth, heating at 70°C for 20 min and incubated at 37°C for 24 h. anaerobically (anaerobic jar with anaerocult A sachet moisted with 17 ml D.W.).

1 ml from the previously prepared dilution was inoculated in 3 tubes of Thioglucolate broth, heating at 70°C for 20 min and incubated at 37°C for 24 h. anaerobically.

Isolation and Identification of Campylobacter (Hunt *et al.,* 2001).

Selective enrichment. 1 ml milk sample was added to 9 ml Preston selective enrichment broth which incubated at 43°C for 48 h. in micro-aerophilic atmosphere (anaerobic jar with anaerocult C sachet of micoaerophilic organism moisted with 6 ml D.W.), Oxoid Ltd., Basing Stoke, UK.

Plating on selective medium. 0.1 ml of Preston selective enrichment broth was added on filter type AC (pore size, 0.45 µm) on the surface of Campylobacter agar medium (Columbia Agar Base + Horse Blood + Campylobacter Selective Supplement Cod SR 204 E + Campylobacter Growth Supplement Code SR 084 E) which incubated at 37°C for 2 h. then removed the filter

Table (1): Statistical analytical results of total colony counts/ml in examined raw ewe's and goat's milk samples.

Samples	No. of examined samples	Count/ml			
		Min.	Max.	Mean	S.E.M.±
Ewe's milk	25	1.0×10^2	1.5×10^4	1.9×10^3	0.60×10^3
Goat's milk	25	2.0×10^2	9.0×10^3	1.4×10^3	0.47×10^3

Table (2): Statistical analytical results of psychrotrophic bacteria counts/ml in examined raw ewe's and goat's milk samples.

Samples	No. of examined samples	Positive samples		Count/ml			
		No.	%	Min.	Max.	Mean	S.E.M.±
Ewe's milk	25	25	100.0	1.5 x10	9.0×10^2	7.8 x10	3.5 x10
Goat's milk	25	25	100.0	1.3 x10	8.1×10^2	6.3 x10	3.1 x10

Table (3): Statistical analytical results of Staphylococci counts/ml in examined raw ewe's and goat's milk samples.

Samples	No. of examined samples	Positive samples		Count/ml			
		No.	%	Min.	Max.	Mean	S.E.M.±
Ewe's milk	25	13	52.0	1.0 x10	4.7×10^2	7.2 x10	2.5 x10
Goat's milk	25	21	84.0	1.0 x10	4.4×10^2	6.1 x10	2.5 x10

Table (4): Statistical analytical results of Enterococci counts/ml in examined raw ewe's and goat's milk samples.

Samples	No. of examined samples	Positive samples		Count/ml			
		No.	%	Min.	Max.	Mean	S.E.M.±
Ewe's milk	25	11	44.0	1.0 x10	9.0 x10	2.5 x10	0.47 x10
Goat's milk	25	9	36.0	1.0 x10	4.0 x10	2.4 x10	0.23 x10

Table (5): Statistical analytical results of *E. coli* counts/ml in examined raw ewe's and goat's milk samples.

Samples	No. of examined samples	Positive samples		Count/ml			
		No.	%	Min.	Max.	Mean	S.E.M.±
Ewe's milk	25	9	36.0	1.0 x10	7.0 x10	3.0 x10	0.41 x10
Goat's milk	25	11	44.0	1.0 x10	5.0 x10	2.1 x10	0.32 x10

Table (6): Incidence of isolated bacterial stains in examined raw ewe's and goat's milk samples.

Isolated bacterial stains	Ewe's milk		Goat's milk	
	No.	%	No.	%
Staph. aureus	9	24.3	10	19.2
Staph. epidermidis	6	16.2	17	32.7
Entrococcus faecalis	4	10.8	7	13.5
Entrococcus faecium	7	19.0	9	17.3
E. coli	11	29.7	9	17.3
Clostridium perfringens	0	0.0	0	0.0
Campylobacter jejuni	0	0.0	0	0.0
Corynebacterium bovis	0	0.0	0	0.0
Salmonellae	0	0.0	0	0.0

and the media were incubated at 43°C for 48 h. microaerophilic in anaerobic jar.

Isolation and Identification of Corynebacterium. 0.1 ml from the previously prepared dilutions of the examined samples was spread onto blood agar plates (5 % defibrinated sheep blood). Plates were incubated aerobically at 37°C and examined after 24 h.

Identification of isolated organisms. Purified colonies were identified by using colony morphology, gram staining characteristics, oxidase, Catalase, coagulase production and biochemical reactions. Specific identifications were made using Commercial micro methods (API Staph for Staphylococci, API 20 Strept for Enterococci, API 20 E for *E. coli*, API 20 A for Clostridia, API Campy, and API Coryne, Bio Merieux, France). Specific serological tests were conducted for Salmonellae spp.: polyvalent (I or II) and monovalent.

Results and Discussion

Results listed in Table (1) revealed that the total bacterial counts/L of examined ewe's and goat's milk samples were ranged from 1.0 $x10^2$ to 1.5 $x10^4$ and 2.0 $x10^2$ to 9.0 $x10^3$ with mean values of 1.9 $x10^3$ and 1.4 $x10^3$, respectively. Higher total bacterial count in ewe's milk was obtained by (Abo-Elnaga *et al.,* 1985) while, higher total bacterial counts in both types were reported in goat's milk (Roberts, 1985; Faschino *et al.,* 2002; Muehlherr *et al.,* 2003; Zweifel *et al.,* 2005). The lower value of total bacterial count in goat's milk was obtained by (Zeng and Escobar 1996).

From the previously listed results, it is observed that any problem with ewe's and goat's milk may be related to poor hygiene during the production rather than transmission of organisms from the animal itself.

Results presented in Table (2) showed that psychrotrophic bacterial counts/ml in examined samples of ewe's and goat's milk were ranged from 1.5 x10 to 9.0 $x10^2$ and 1.3 x10 to 8.1 $x10^2$ with mean values of 7.8 x10 and 6.3 x10, respectively. The presence of large numbers of psychrotrophic organisms is not necessarily indicative of an immediate health hazard because pasteurisation kills virtually all of the thermolabile psychrotrophs but it does indicate a lack of good sanitary practices where the growth and metabolic activity originating from post pasteurisation contamination that give rise to spoilage, loss of quality or create a health hazard (James *et al.,* 1973; Sorhaug and Stepaniak, 1997).

The results summarized in Table (3) decleared that the Staphylococcus spp. could be detected in 52.0 and 84.0 % of examined ewe's and goat's milk samples with mean values of 7.2 x10 and 6.1 x10, respectively. Lower percentages and lower counts/ml were reported (Abo-Elnaga *et al.,* 1985; Roberts, 1985; Little and De Louvois, 1999; Muehlherr *et al.,* 2003) while, higher counts were reported by (Faschino *et al.,* 2002; Holeckova *et al.,* 2004). The main isolated Staphylococci strains were *Staphylococcus aureus* and *Staphylococcus epidermidis* at percentages of (24.3 & 19.2 %) and (16.2 & 32.7 %) of total isolates, respectively (Table, 6). Highest Staphylococci count is good indication of clinical udder alteration Deinhofer and Pernthaner, 1993 because one of the important causes of mastitis either in ewes or goats is staphylococci (Smith and Roguinsky, 1977).

The results tabulated in Table (4) revealed that the enterococci could be detected in 44.0 and 36.0 % of examined ewe's and goat's milk samples with mean values of 2.5 x10 and 2.4 x10, respectively. Higher enterococci counts were reported by (Little and De Louvois, 1999; Faschino *et al.,* 2002). The predominant isolated enterococci strains were *Enterococcus faecalis* and *Enterococcus faecium* at percentages of (10.8 &13.5 %) and (19.0 &17.3 %) of total isolates, respectively (Table, 6).

Table (5) showed that *E. coli* could be isolated from 36.0 and 44.0 % of examined ewe's and goat's milk samples. The *E. coli* counts/ml ranged from 1.0 x10 to 7.0 x10 and 1.0 x10 to 5.0 x10, with mean counts of 3.0 x10 and 2.1 x10, respectively. Isolated *E. coli* strains of examined ewe's and goat's milk samples represented 29.7 and 17.3 % of total isolates, respectively (Table, 6). *E. coli* failed to be detected by (Little and De Louvois, 1999) while, *E. coli* was detected in one sample with a very lower count (Faschino *et al.,* 2002; Dontorou *et al.,* 2003). Higher prevalence was reported by Roberts, (1985) and lower percentage was detected by Rey *et al.,* (2006).

From the obtained results we found that the prevalence of *E. coli* in goat's milk is higher than that of ewe's milk and this finding is in agreement with those reported by (Muehlherr *et al.,* 2003). Accordingly, goats may act as a reservoir of *E. coli* and goat's milk as well as dairy products may serve as vehicle for the pathogen transmission to humans.

The results listed in Table (6) revealed that the *Clostridium perfringens*, *Campylobacter jejuni*, *Corynebacterium bovis* and Salmonellae failed to be detected in any of examined samples of ewe's and goat's milk. Similar finding were reported by(Abo-Elnaga *et al.,* 1985; Faschino *et al.,* 2002; Muehlherr *et al.,* 2003). While, Roberts, (1985) could detect Campylobacter jejuni in only one samples.

Conclusion

It is concluded that the differences in count between ewe's and goat's milk may be related to the species differences as well as milking methods. Contamination of milk can be eliminated by following strict hygienic production measures and pasteurization where pasteurization largely eliminates this hazard.

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النوعية البكتيرية للبن النعاج والماعز الخام متضمنة ميكروبات التسمم الغذائي

تم تجميع خمسون عينة من لبن النعاج والماعز الخام (٢٥ خمسة وعشرون عينة من كل نوع) من مزرعة ليندنهوف – مدينة شتوتجارت – ألمانيا. وفحصها بكتريولوجيا بمعهد الصحة والبيئة بجامعة هوهنهايم لمعرفة تواجد العدد الكلي للميكروبات والميكروبات المحبة للبرودة بالإضافة إلي دراسة تواجد ميكروبات التسمم الغذائي بها. وقد أوضحت الدراسة أن متوسط العدد الكلي للميكروبات في العينات المفحوصة كان ١،٩×١٠^٣ و ١،٤×١٠^٣ / مللي علي التوالي. كما أمكن عزل الميكروبات المحبة للبرودة من جميع العينات (١٠٠ %) بمتوسط ٧،٨ ×١٠و ٦،٣ ×١٠ / مللي علي التوالي. قد أمكن عزل كلا من ميكروبات المكورات العنقودية والمكورات السبحية المعوية وميكروب الإيشيريشيا كولاي بنسبة (٥٢ و ٨٤ %) و (٤٤ و ٣٦ %) و (٣٦ و ٤٤ %) وكان متوسط العدد الكلي للمعزولات هو (٧،٢ ×١٠و ٦،١ ×١٠) و (٢،٥ ×١٠و ٢،٤ ×١٠) و (٣×١٠ و ٢،١ ×١٠) لكل مللي من عينات لبن النعاج والماعز علي التوالي وبتصنيف المعزولات أمكن الحصول علي عترات المكور العنقودي الذهبي و المكور العنقودي من نوع إبيدرميدس و المكور السبحي المعوي من نوع فيكالس و من نوع فيشيوم وميكروب الإيشيريشيا كولاي بنسبة (٢٤،٣ و ١٩،٢ %) و (١٦،٢ و ٣٢،٧ %) و (١٠،٨ و ١٣،٥ %) و (١٩،٠ و ١٧،٣ %) و (٢٩،٧ و ١٧،٣ %) من إجمالي المعزولات علي التوالي. في حين أن ميكروبات كلوستريديم بيرفرنجينز و كامبيلو باكتر جيجيناي و كوريني بكتريم بوفيز السالمونيلا لم يتمكن من عزلها من أي من العينات المفحوصة. وكذلك تمت مناقشة الأهمية الصحية للمعزولات وكيفية تطبيق الأسس العلمية لمنع انتقال هذه الميكروبات للإنسان عن طريق تناول لبن النعاج والماعز للحفاظ علي صحة المستهلك.