Journal of Dairy Research (2002) **69** 213–225. © *Proprietors of Journal of Dairy Research 2002*
DOI: 10.1017/S0022029902005459 *Printed in the United Kingdom*

Microbial composition, including the incidence of pathogens, of goat milk from the Bergamo region of Italy during a lactation year

By ROBERTO FOSCHINO[1]*, ALESSANDRO INVERNIZZI[2], ROBERTO BARUCCO[1] and KATIA STRADIOTTO[3]

[1] *Università degli Studi di Milano, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, via G. Celoria 2-20133 Milano, Italy*
[2] *Caseificio Giovanni Invernizzi, via Mazzini 40-24040 Pontirolo Nuovo (BG)*
[3] *Associazione Regionale Allevatori, via J. F. Kennedy 30-26013 Crema (CR)*

(*Received 5 March 2001 and accepted for publication 2 October 2001*)

Summary. Sixty samples of raw goat milk intended for Caprino cheese-making were collected from ten farms in the Bergamo area over a 6-month period. Analyses of main microbial groups, somatic cell count (SCC) and pH were performed to determine the effect of origin (farm) and lactation period (April – September) on microbial composition and the incidence of pathogens in milk. Overall mean values were: standard plate count (SPC), $5{\cdot}0 \times 10^4$ cfu/ml; yeasts, $2{\cdot}5 \times 10^2$ cfu/ml; coliforms, $9{\cdot}1 \times 10^2$ cfu/ml; *Escherichia coli*, 2·9 cells/ml; enterococci, $1{\cdot}1 \times 10^2$ cfu/ml; lactococci, $3{\cdot}4 \times 10^3$ cfu/ml; lactobacilli, $3{\cdot}0 \times 10^3$ cfu/ml; halotolerant bacteria, $8{\cdot}2 \times 10^3$ cfu/ml; spores of mesophilic aerobic bacteria, 11 cfu/ml; SSC, $9{\cdot}9 \times 10^5$ cells/ml; pH, 6·63. Moulds and spores of sulphite-reducing clostridia were found intermittently. Neither *Salmonella* spp. nor *Listeria monocytogenes* was detected, while *Esch. coli* O157:H7 was isolated from one milk sample (an incidence of 1·7 %). *Staphylococcus aureus* was discovered at a level $>10^2$ cfu/ml in 26 samples (43 %) with an overall mean of $1{\cdot}2 \times 10^3$ cfu/ml, whereas coagulase-negative staphylococci were found in 54 samples (90 %) with an overall mean of $1{\cdot}3 \times 10^3$ cfu/ml. Of *Staph. aureus* strains, 23 % proved to be enterotoxinogenic with a prevalence of enterotoxin C producers. *Staph. caprae* was the coagulase-negative species most frequently isolated; none of the coagulase-negative staphylococci strains synthesized any of the enterotoxins tested for.

Sample source was the major factor affecting the microbial composition of goat milk: significant differences ($P < 0{\cdot}01$) were observed among samples from different farms for SPC, coliforms, lactococci, lactobacilli and halotolerant bacteria. Period of lactation had a significant effect ($P < 0{\cdot}025$) on SCC and pH. SPC correlated well with coliforms, lactococci and lactobacilli; SSC did not reveal positive interactions with any microbial groups or pH.

Keywords: goat milk, microflora, pathogens.

Manufacture of dairy products from milk of small ruminants presents an opportunity to improve the economics of breeding and cheese-making in remote areas such as mountainous regions. Safeguarding and promoting traditional goat products has become part of a policy directed towards the protection of the

* For correspondence; e-mail: Roberto.Foschino@unimi.it

environment and preserving the social culture of the Alpine territories. This objective would benefit from knowledge of the economic, animal production, hygienic and technological aspects of the manufacture of such products.

Traditional Caprino cheese, a typical fresh goat-cheese obtained by natural acid-curdling, is mostly made on dairy farms of Piedmont and Lombardy from raw milk, whose microflora benefits the sensory characteristics of the final products (Foschino *et al*. 1999). At the same time, if pathogens survive or develop in cheese, they can be a hazard to consumers. Information available on the microbiological quality of goat milk (Lodi, 1993) and cheeses in Italy (Frontini, 1997) is not sufficient to demonstrate the suitability and safety of the technology used in the preparation of Caprino cheese. Milk is not heat-treated and, indeed, it would be difficult to carry out, because of the economics of small-scale production. Therefore, improving hygienic conditions during milking is the principal point at which to control microbiological hazards (Lodi *et al*. 1994).

This work, carried out over a lactation year, was aimed at determining microbial composition, and the incidence of pathogens (*Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and their toxins), in raw goat milk intended for cheese-making, to determine the influence of farm of origin and lactation period.

Materials and methods

Selection of farms

Ten farms located in Lombardy were selected on the basis of their herd sizes (10–120 milking goats), geographical area (around Bergamo and its Alpine region), type of feeding (grass, hay and/or forage crops), type of production (raw milk for manufacture of Caprino cheese) and willingness of the breeders to collaborate in the research (Table 1). All herds were housed in collective cabins with litter of straw or wood shavings replaced monthly. During summer, goats of farms E and H were led to pasture. Breeder E treated the herd with homoeopathic therapy. All farms milked twice daily, at 12-h intervals, using milking machines except for farm B, on which goats were milked by hand. Raw milk was refrigerated after milking, except for farms B and H. Only the herd of farm H tested negative for caprine arthritis encephalitis virus (CAEV) probably because that herd was made up solely of goats belonging to the Orobic breed, an indigenous race of the Alpine region of Lombardy not yet infected at an endemic level. Other characteristics of the farms are reported in Table 1.

Sample collection and treatment

Samples (500 ml) of raw milk were collected monthly from April to September, inclusive. Each sample was taken aseptically from the on-farm refrigerated tanks (except for farms B and H where tanks were at room temperature), stored at 4 °C and analysed within 24 h. Samples were prepared for microbiological examination according to International Dairy Federation (IDF) Standard 122B:1992 (1992).

Microbiological analysis

SPC was determined according to IDF Standard 100B:1991(1991). Yeasts and moulds were counted according to IDF Standard 94B:1990 (1990). Coliform counts were performed according to IDF Standard 73B:1998 Part 1, Colony count technique at 30 °C (1998).

Esch. coli concentration was measured according to IDF Standard 170:1994 (1) Most probable number technique (1994). For *Esch. coli* O157, two enrichment

Table 1. *Characteristics of the ten farms from which milk samples were obtained*

(Values are means ± SD)

Farm	Breed	Number in herd	Milking method	Daily milk production (l/goat)
A	Alpine	21	Bucket	3·6 ± 0·7
B	Alpine	10	Hand	3·0 ± 0·6
C	Saanen	55	Pipeline	2·8 ± 0·1
D	Alpine†	120	Bucket	2·9 ± 0·4
E	Alpine	46	Pipeline	3·4 ± 0·6
F	Saanen	45	Bucket	3·1 ± 0·6
G	Saanen	38	Bucket	3·6 ± 0·9
H	Orobic	25	Bucket	1·7 ± 0·4
I	Orobic‡	38	Pipeline	2·1 ± 0·6
L	Saanen	29	Pipeline	2·7 ± 0·6

† Containing a minority of Saanen.
‡ Containing a minority of Alpine.

methods were used. The first consisted of inoculation of 25 ml milk into 225 ml of modified EC selective broth (1·12 g/l bile salts no. 3, 20 mg/l Novobiocin) and incubation at 37 °C for 24 h. A loop of the culture was streaked on MacConkey Sorbitol agar (March & Ratnam, 1986) containing Cefixime (0·05 mg/l) and potassium tellurite (2·5 mg/l; CT-SMaC) and incubated at 37 °C for 24 h. Sorbitol-negative colonies were grown on Tryptone Soy agar, identified by API 20E (BioMérieux, Marcy l'Etoile, France) and tested with *Esch. coli* antisera O157 and H7 (Difco, Detroit, Michigan, USA) for serological identification. The second method provided for a selective enrichment of a 25-ml sample, followed by an enzyme-linked fluorescent immunoassay (VIDAS ECO, BioMérieux, Marcy l'Etoile, France) for the detection of O157 antigen, carried out in an automated miniVIDAS system (BioMérieux, Marcy l'Etoile, France) as specified by the supplier.

For enterococci, suitable dilutions of samples were plated on Kanamycin Aesculin Azide agar and then incubated at 45 °C for 24 h. Incubation temperature was increased relative to the original description (Mossel *et al.* 1978) to improve the selectivity of the medium.

For lactococci, suitable dilutions were plated on M17 agar (Terzaghi & Sandine, 1975) and then incubated at 30 °C for 72 h. Sodium azide (75 mg/l) was added to the medium to inhibit growth of microorganisms with respiratory metabolism. Data reported as ''lactococci'' are the result of subtraction of counts obtained in Kanamycin Aesculin Azide agar from counts obtained in M17 agar since enterococci still grow in this medium.

Lactobacilli were plated on MRS agar (DeMan *et al.* 1960), pH 5·4, and then incubated at 30 °C for 72 h in an anaerobic atmosphere (GasPak System, BBL, Kansas City, Missouri, USA).

Halotolerant bacteria were quantified as microflora able to grow in 7·5 % NaCl (Micrococcaceae), suitable dilutions of samples were plated on Mannitol Salt agar (Chapman, 1945) and incubated at 30 °C for 48 h.

Staphylococcus aureus and other staphylococci were plated on Baird-Parker agar and then incubated at 37 °C for 48 h (IDF Standard 145A : 1997). Black colonies > 1 mm in diameter were counted separately on the basis of their morphological characteristics and, for each typology of colony, were isolated and identified. The following tests were performed : Gram staining, catalase reaction, coagulase test with rabbit plasma fibrinogen (Bactident Coagulase, Merck, Darmstadt, Germany),

haemolysis test in Columbia Agar with sheep defibrinated blood (Oxoid, Unipath, Basingstoke, UK) and API ID 32 Staph (BioMérieux, Marcy l'Etoile, France). The capacity of each strain to produce enterotoxins was evaluated by reversed passive latex agglutination test SET RPLA (Oxoid, Unipath, Basingstoke, UK) after growth of the cultures in Tryptone Soy broth at 37 °C for 24 h.

For counting spores of mesophilic aerobic bacteria, milk samples (10 ml) were first heated in a water bath (80 °C for 10 min) to eliminate the non-spore-forming microflora and vegetative cells and then suitable dilutions were plated on Plate Count agar and incubated at 30 °C for 48 h.

Spores of sulphite-reducing clostridia were determined in suitable dilutions of samples previously heated at 80 °C for 10 min, as described above, and inoculated in tubes containing melted Sulphadiazine Polymixine Sulphite agar (Angelotti *et al.* 1962). Incubation was at 37 °C for 48 h.

Two isolation techniques were used for *Salmonella* spp. The first was that described in IDF Standard 93B:1995 (1995). The second technique involved an enrichment protocol starting from a 25-ml sample, followed by an enzyme-linked fluorescent immunoassay (VIDAS SLM, BioMérieux, Marcy l'Etoile, France) for the detection of *Sal.* antigens, performed in an automated miniVIDAS system.

For *Listeria monocytogenes*, two isolation techniques were used. The first was that described in IDF Standard 143A:1995 (1995). The second technique provided for a selective enrichment of a 25-ml sample followed by an enzyme-linked fluorescent immunoassay (VIDAS LMO, BioMérieux, Marcy l'Etoile, France) for the detection of *List. monocytogenes* antigens, carried out in an automated miniVIDAS system.

Sample pH was determined using a pH meter (Gibertini, Milan, Italy). For SCC, a laser beam Bentley Somacount 150 (Bentley Instruments, Chaska, Minnesota, USA) was used.

Statistical analysis

Results of microbiological and SCC were transformed into respective decimal logarithms to fit a normal distribution of values. Data were subjected to one-way ANOVA (Camussi *et al.* 1986) to investigate separately the effect of sample origin (farm) and period of sampling (month). When the effect was significant ($P < 0{\cdot}05$), differences between means were separated by Tukey's test of multiple comparisons. Correlation coefficients among different variables were also calculated by the general linear model.

RESULTS

Monitoring of the microflora of milk by farm

Table 2 shows log counts in raw milk samples for each farm. SPC was always below the limit fixed by EU regulations (European Council Directive 92/46/ECC 1992); only five samples between June and August (two collected at farm B, two at D and one at H) exceeded $5{\cdot}0 \times 10^5$ cfu/ml. The overall mean was $5{\cdot}0 \times 10^4$ cfu/ml; significant differences ($P = 0{\cdot}001$) were observed among SPCs, with higher values in samples from farms A, B and D. Yeasts were regularly found (95% of samples) with an overall mean value of $2{\cdot}5 \times 10^2$ cfu/ml, while moulds were detected in only 48% of samples, with an overall mean value of $1{\cdot}1 \times 10^2$ cfu/ml. Coliforms were a constant component of the microflora with an overall mean of $9{\cdot}1 \times 10^2$ cfu/ml; samples from farms B and H showed significantly higher counts ($P = 0{\cdot}02$). Enterococci were found at a steady level (overall mean $1{\cdot}1 \times 10^2$ cfu/ml) without noteworthy differences among samples. Lactococci and lactobacilli were permanent components

https://doi.org/10.1017/S0022029902005459 Published online by Cambridge University Press

Table 2. *Mean log counts (per ml) of microorganisms, somatic cell counts (SSC) and pH values in raw goat milk samples from the various farms*

(Values are means and SD for $n = 6$)

Farm		Standard plate Count	Yeasts	Coliforms	Enterococci	Lactococci	Lactobacilli	Halotolerant bacteria	SCC	pH
A	Mean	$5{\cdot}256^{ab}$	$2{\cdot}143^{ab}$	$2{\cdot}515^{a}$	2·220	$3{\cdot}780^{ab}$	$3{\cdot}985^{a}$	$3{\cdot}637^{ab}$	$6{\cdot}162^{a}$	6·65
	SD	0·332	0·824	0·459	1·029	1·183	0·626	0·429	0·262	0·10
B	Mean	$5{\cdot}670^{a}$	$2{\cdot}526^{ab}$	$4{\cdot}333^{b}$	1·883	$3{\cdot}569^{abc}$	$4{\cdot}312^{a}$	$4{\cdot}362^{a}$	$6{\cdot}026^{ab}$	6·59
	SD	0·451	0·534	0·689	0·444	0·861	0·578	0·298	0·505	0·04
C	Mean	$4{\cdot}702^{bc}$	$2{\cdot}250^{ab}$	$2{\cdot}986^{a}$	2·137	$3{\cdot}827^{ab}$	$4{\cdot}130^{a}$	$3{\cdot}697^{ab}$	$6{\cdot}002^{ab}$	6·63
	SD	0·212	0·563	0·433	0·476	0·465	0·399	0·635	0·163	0·06
D	Mean	$5{\cdot}312^{ab}$	$2{\cdot}771^{ab}$	$2{\cdot}814^{a}$	2·873	$4{\cdot}440^{a}$	$4{\cdot}392^{a}$	$4{\cdot}196^{a}$	$6{\cdot}247^{a}$	6·68
	SD	0·821	0·511	0·375	0·962	0·551	0·595	0·288	0·090	0·03
E	Mean	$4{\cdot}107^{c}$	$1{\cdot}884^{a}$	$2{\cdot}651^{a}$	2·488	$3{\cdot}315^{abc}$	$2{\cdot}498^{b}$	$4{\cdot}306^{a}$	$5{\cdot}976^{ab}$	6·59
	SD	0·238	0·589	0·328	0·652	0·686	0·579	0·077	0·076	0·07
F	Mean	$4{\cdot}317^{c}$	$2{\cdot}987^{b}$	$2{\cdot}828^{a}$	2·408	$2{\cdot}484^{c}$	$2{\cdot}600^{b}$	$3{\cdot}311^{b}$	$5{\cdot}975^{ab}$	6·63
	SD	0·554	0·779	0·677	1·347	0·627	0·638	0·656	0·331	0·11
G	Mean	$4{\cdot}329^{c}$	$2{\cdot}463^{ab}$	$2{\cdot}437^{a}$	1·559	$3{\cdot}588^{abc}$	$2{\cdot}463^{b}$	$3{\cdot}951^{ab}$	$6{\cdot}051^{ab}$	6·61
	SD	0·411	0·389	0·572	0·605	0·347	0·389	0·334	0·139	0·07
H	Mean	$4{\cdot}716^{bc}$	$2{\cdot}357^{ab}$	$3{\cdot}163^{ab}$	1·721	$3{\cdot}753^{ab}$	$3{\cdot}809^{a}$	$4{\cdot}137^{a}$	$5{\cdot}617^{b}$	6·61
	SD	0·633	0·591	1·375	0·307	0·338	0·832	0·314	0·270	0·11
I	Mean	$4{\cdot}558^{bc}$	$2{\cdot}720^{ab}$	$2{\cdot}935^{a}$	1·560	$3{\cdot}670^{abc}$	$3{\cdot}779^{a}$	$3{\cdot}930^{ab}$	$6{\cdot}078^{ab}$	6·60
	SD	0·374	0·432	0·791	0·499	0·266	0·510	0·532	0·370	0·05
L	Mean	$3{\cdot}996^{c}$	$1{\cdot}822^{a}$	$2{\cdot}935^{a}$	1·711	$2{\cdot}856^{bc}$	$2{\cdot}796^{b}$	$3{\cdot}616^{ab}$	$5{\cdot}826^{ab}$	6·69
	SD	0·326	0·322	0·601	1·041	0·533	0·660	0·132	0·213	0·09

^{a,b,c} Values in the same column with different superscripts differ significantly ($P < 0{\cdot}05$).

of the microflora with overall means of $3{\cdot}4 \times 10^3$ cfu/ml and $3{\cdot}0 \times 10^3$ cfu/ml, respectively. For both groups, significant differences ($P = 0{\cdot}001$) were observed among samples collected from different farms. Also counts of halotolerant bacteria (overall mean $8{\cdot}2 \times 10^3$ cfu/ml) showed significant differences ($P = 0{\cdot}001$) with higher values in samples from farms B, D, E and H. Spores of mesophilic aerobic bacteria were present in 87 % of samples, at a low level (overall mean of 11 cfu/ml) whereas spores of sulphite-reducing clostridia were demonstrated in only 13 % of samples (overall mean of 1·1 cfu/ml). Overall mean SCC was $9{\cdot}9 \times 10^5$ cells/ml with 47 % of samples higher than 1×10^6 cells/ml; SCC in milk collected from farms A and D were significantly different from those from H ($P = 0{\cdot}022$). The pH ranged between 6·49 and 6·80, with an overall mean of 6·63; there were no variations among samples from different farms.

Monitoring of the microflora of milk by period of lactation

Table 3 shows log counts in raw milk samples for each month. Changes in SPC were not significantly affected by period of sampling. In April and May, moulds were detected only in milk from two farms, while in June and September they were detected in six. Eight out of ten samples contained moulds in July and August with mean values of $8{\cdot}7 \times 10^1$ and $6{\cdot}5 \times 10^1$ cfu/ml, respectively. Counts of yeasts, coliforms, enterococci, lactococci, lactobacilli and halotolerant bacteria were not significantly different in samples collected in different months. Also the levels of spores of mesophilic bacilli and sulphite-reducing clostridia appeared not to be influenced by season. For SCC, significant differences ($P = 0{\cdot}022$) were observed: the minimum mean value was detected in June ($6{\cdot}5 \times 10^5$ cells/ml) and the maximum in September ($1{\cdot}6 \times 10^6$ cells/ml). Values of pH showed significant differences ($P = 0{\cdot}007$) among samples collected in different months, with decreasing values from April (6·69) to September (6·56); the overall mean was 6·63.

Detection of Esch. coli *and* Esch. coli *O157*

Overall mean count of *Esch. coli* was 2·9 cells/ml, taking into account only those samples that contained $\geqslant 0{\cdot}3$ cells/ml (54 out of 60); only 4 samples (7 %) exceeded 100 cells/ml. Data separated by farm showed a minimum mean value of 0·5 cells/ml in samples from farm E and a maximum of 7·9 cells/ml in samples from farm C. Data grouped by month showed higher mean values in samples collected in summer time, 6·7 cells/ml in June, 3·6 cells/ml in July and 4·5 cells/ml in August.

To isolate *Esch. coli* O157, two techniques were used in parallel. The first (CT-SMaC) did not recover any *Esch. coli* O157 strains. Sorbitol-negative colonies were isolated in 16 samples but strains were identified as *Esch. coli* not O157 (26 %), *Pseudomonas aeruginosa* (26 %), *Pseudomonas* spp. (21 %) and other Gram negative bacteria (27 %). The second technique (VIDAS-ECO) revealed one positive sample of *Esch. coli* O157, from D farm in July. Biochemical and serological tests confirmed the identification of this strain as *Esch. coli* O157:H7. In this sample, the *Esch. coli* concentration was 1·5 cells/ml.

Detection of other pathogens

No *Salmonella* spp. were detected in any milk sample using the IDF protocol (IDF Standard 93B:1995), whereas two samples gave positive results with the VIDAS system (VIDAS SLM). Serological and biochemical tests on isolates from the relevant enrichment broths did not confirm their identification as being of the genus *Salmonella*, and instead they were ascribed to *Enterobacter* or *Enterococcus cloacae*.

Table 3. *Mean monthly log counts (per ml) of microorganisms and somatic cell count (SCC) and pH values in samples of raw goat milk*

(Values are means and SD for $n = 10$)

Month		Standard plate count	Yeasts	Coliforms	Enterococci	Lactococci	Lactobacilli	Halotolerant bacteria	SCC	pH
April	Mean	4·572	2·187	2·629	2·704	3·041	3·312	3·982	$5·855^{ab}$	$6·69^{a}$
	SD	0·853	0·379	0·841	0·920	0·772	0·807	0·306	0·206	0·05
May	Mean	4·764	2·588	2·881	2·227	3·530	3·499	4·067	$5·959^{ab}$	$6·67^{a}$
	SD	0·547	0·587	0·544	0·570	0·848	0·842	0·433	0·329	0·07
June	Mean	4·993	2·670	3·547	1·868	3·644	3·776	3·860	$5·812^{a}$	$6·62^{ab}$
	SD	0·966	0·465	1·110	1·045	0·949	1·353	0·688	0·314	0·09
July	Mean	4·637	2·654	3·098	1·684	3·489	3·529	4·015	$6·125^{ab}$	$6·62^{ab}$
	SD	0·391	0·747	0·697	0·623	0·616	0·648	0·423	0·324	0·09
August	Mean	4·655	2·285	2·783	1·950	3·690	3·497	3·690	$6·027^{ab}$	$6·61^{ab}$
	SD	0·627	0·703	0·695	0·946	0·898	0·948	0·524	0·315	0·07
September	Mean	4·558	1·969	2·820	1·904	3·775	3·244	3·873	$6·198^{b}$	$6·56^{b}$
	SD	0·653	0·684	0·744	0·691	0·522	1·004	0·586	0·142	0·06

[a,b] Values in the same column with different superscripts differ significantly ($P < 0·05$).

Table 4. *Correlation coefficients among the variables measured in the samples of raw goat milk*

	SPC	Coliforms	*Esch. coli*	Enterococci	Halotolerant bacteria	Coag.-positive staphylococci	Coag.-negative staphylococci	Yeasts	Lactococci	Lactobacilli	Somatic cells	pH
Observations (n)	60	60	54	60	60	25	54	57	60	60	60	60
SPC	1·000	0·558**	0·025	0·186	0·301*	0·422*	0·045	0·290*	0·508**	0·722**	0·016	−0·067
Coliforms		1·000	0·394**	0·177	0·231	0·475*	−0·102	0·301*	0·116	0·369**	−0·231	−0·190
Esch. coli			1·000	−0·264	−0·100	0·107	−0·017	0·081	−0·108	−0·032	−0·262	−0·304*
Enterococci				1·000	0·050	−0·138	0·189	−0·017	−0·066	0·272	−0·154	0·189
Halotolerant bacteria					1·000	0·378	0·177	0·061	0·232	0·177	−0·035	−0·091
Coag.-positive staphylococci					1·000	−0·090	0·361	0·068	0·412*	0·182	−0·202	
Coag.-negative staphylococci							1·000	−0·168	0·136	−0·050	−0·096	−0·117
Yeasts								1·000	0·202	0·146	0·122	0·005
Lactococci									1·000	0·491**	0·132	−0·137
Lactobacilli										1·000	−0·011	0·034
SCC											1·000	−0·229
pH												1·000

Significance levels: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

List. monocytogenes was not revealed in any milk sample, either with the IDF protocol (IDF Standard 143A:1995) or the VIDAS system (VIDAS LMO).

Detection of Staph. aureus *and other staphylococci, identification of the strains and enterotoxin synthesis*

Staph. aureus was detected at a level > 100 cfu/ml in 26 samples (43 %) with an overall mean of $1{\cdot}2 \times 10^3$ cfu/ml. Mean values in raw milk collected from two farms (B and D, $7{\cdot}3 \times 10^3$ and $2{\cdot}4 \times 10^3$ cfu/ml, respectively) exceeded the maximum limit of the European Council Directive 92/46/ECC (1992) for raw goat milk for cheese-making. On the other hand, no sample from farms A and H had > 100 cfu/ml. All strains, identified as *Staph. aureus* by API ID 32 Staph test, were haemolytic (43 % distinctly α-haemolytic and 57 % α/β haemolytic); 97 % (29 strains out of 30) were coagulase-positive, whereas only 40 % (12 out of 30) were lecithinase-positive. Seven strains (23 %) were able to synthesize enterotoxins: five strains produced enterotoxin C, one strain produced enterotoxin D and one strain produced enterotoxin A.

Coagulase-negative staphylococci were detected at a level > 100 cfu/ml in 54 samples (90 %) with an overall mean of $1{\cdot}3 \times 10^3$ cfu/ml; the minimum mean value ($6{\cdot}4 \times 10^2$ cfu/ml) was found in milk from farm C and the maximum mean value ($9{\cdot}0 \times 10^3$ cfu/ml) in milk from farm E. During the lactation period the numbers of coagulase-negative staphylococci increased from April ($8{\cdot}5 \times 10^2$ cfu/ml) to September ($4{\cdot}5 \times 10^3$ cfu/ml). Of the 74 strains isolated, 28 (38 %) were identified as *Staph. caprae*, 7 (9 %) as *Staph. chromogenes*, 7 (9 %) as *Staph. xylosus*, 5 (7 %) as *Staph. epidermidis*, 3 (4 %) as *Staph. saprophyticus*, 3 (4 %) as *Staph. simulans*, 2 (3 %) as *Staph. equorum* and the remaining 19 (26 %) to other species of *Staphyloccus* or *Micrococcus*. None of the coagulase-negative staphylococcal strains examined produced any of the enterotoxins tested for.

Correlation among microbial groups, somatic cells counts and pH

Analyses of correlation coefficients among components of microflora, SCC and pH are summarized in Table 4. SPC showed positive interactions with coliforms, halotolerant bacteria, yeasts and lactic acid bacteria, so that they exhibit a constant part of the microflora recovered as total count in raw milk. Coliforms showed significant positive correlation with *Esch. coli*, coagulase-positive staphylococci, yeasts and lactobacilli; furthermore, lactobacilli also correlated with lactococci and enterococci. On the other hand, staphylococci coagulase-negative and somatic cells did not correlate with any microbial groups or pH.

DISCUSSION

Factors affecting the microbiological quality of raw goat milk

Sample origin (i.e. farm) was the main factor affecting the microbial composition of the goat milk. This finding may result from the sum of different elements, such as type of feeding, hygienic conditions of milking, breeders' practices and location of farm. Actually, the proportional contribution of a particular microbial group to the total count in milk was consistent for samples collected from the same farm, whereas it varied considerably for samples from different producers. If SPCs are compared with the levels of each microbial group, the microflora of milk from farm E, where the breeder employed homoeopathic products, consisted almost solely of Micrococcaceae. Similarly the microflora of milk from farms G and L was composed mainly of halotolerant bacteria (50 %) and lactic acid bacteria (25 %). In the case of milk

from farms A, B and D, over 70% of the microflora remains unassigned; this may be ascribed to pseudomonads (Desmasures & Gueguen, 1997). Moreover, SPCs of milk from farms E, F, G and L, which had lower counts and a predominance of Gram-positive bacteria, were significantly different from SPCs of milk from farms A, B and D, which had higher counts and a prevalence of Gram-negative bacteria.

Numbers of yeasts and moulds are similar to those reported by Tirard-Collet *et al.* (1991); fungal counts are higher in farms without pipelines, suggesting that this type of contamination is likely to be due to airborne dust particles.

Level of coliforms is related to hygiene during production and rapid cooling of milk (White, 1998). Significant differences amongst farms were seen for this group but they were not affected by stage of lactation. Furthermore, the count was significantly higher in samples collected from farms that did not refrigerate the milk (B and H). Notably, coliforms correlated well with SPC and, as expected, with *Esch. coli*. Mean values of *Esch. coli* reported in this work are greater than those found by Tirard-Collet *et al.* (1991) for raw goat milk from Quebec. Nevertheless the occurrence of *Esch. coli* is less than that found by Dumoulin & Peretz (1993) in France. Contamination of *Esch. coli* seems to follow a seasonal distribution with higher values in summer time.

Lactic acid bacteria, as a whole, appear to be an important part of the natural microflora. An overlap between counts of lactococci and lactobacilli probably occurs as the media used are not sufficiently selective to differentiate them accurately. However, enterococci counts emerge distinct from the others. This is supported by analysis of correlation coefficients, which revealed a positive interaction between lactococci and lactobacilli, while enterococci did not correlate with any variable investigated. For this reason, the finding of enterococci in dairy products from raw milk, such as *Caprino* cheese, does not appear to be associated directly with faecal contamination or poor hygiene.

Halotolerant bacteria are recognized to be a stable group in goat milk. *Micrococcus*, rather than *Staphylococcus*, probably constitutes the major genus recovered in this work, given that no positive interactions were found between counts in Mannitol Salt agar and colony numbers of coagulase-positive staphylococci or coagulase-negative staphylococci or the sum of them, grown on Baird-Parker medium.

No relationship was established between SCC and any particular microbial groups or pH values. As reported by others (Tirard-Collet *et al.* 1991; Wilson *et al.* 1995; Zeng & Escobar, 1995, 1996), an increase in SPC, or in a particular bacterial group in milk, does not necessarily correlate with a rise in SCC, which is mainly affected by breed, period of lactation and parity. However, milk from farm H, whose herd was the only one not infected by CAE virus, showed significantly lower SCC.

Lactation period did not affect the level of any microbial group investigated but, on the contrary, SCC and pH were significantly influenced by this factor. These observations agree with the findings of Wilson *et al.* (1995) and Zeng & Escobar, (1995) who identified an increase of SCC as lactation progressed. Decrease of pH is probably linked to an increase of protein concentration in milk at the end of lactation, caused by the natural drop in milking capacity (Zeng & Escobar, 1996).

Incidence of pathogens

Food infections caused by Verotoxigenic *Esch. coli* are emerging as a public health concern (Doyle, 1991; Tarr *et al.* 1997; Coia, 1998) and fresh dairy products made from raw milk could be implicated (Morgan *et al.* 1993; Bielaszewska *et al.* 1997). In

the present work, an *Esch. coli* O157:H7 strain was isolated in one milk sample (1·7%) employing the analytical procedure provided by the VIDAS system. The other cultural method, used in parallel on the same samples, was not able to recover it; in fact, sorbitol negative colonies were not observed on CT-SMaC.

Coliforms can grow to high levels in the manufacture of traditional Caprino cheese during milk curdling and the early ripening period (Foschino *et al.* 1999). Therefore an HACCP system must be rigorously applied to prevent microbiological risks (Lodi *et al.* 1994). Since pH is the only limiting factor in the product to control survival or growth of bacteria, a ripening stage of not less than 10 d is recommended. If the traditional cheese-making process is to be maintained and the typical characteristics of Caprino cheese not lost, it is necessary to improve the microbiological quality of the raw milk (Lodi, 1993; Lodi *et al.* 1994). Technical help to farmhouse-cheese producers and the introduction of a payment scheme based on quality, which already occurs in other countries (Dumoulin & Peretz, 1993), are needed.

Identifying the maximum level of *Staph. aureus* in unpasteurized milk intended for cheese production is necessary because dairy products from raw milk are still frequently implicated in food poisoning (Gilmour & Harvey, 1990; Mossel & Van Netten, 1990). Some reports have established that coagulase-negative species are also able to synthesize enterotoxins (Valle *et al.* 1990; Vernozy-Rozand *et al.* 1996). In addition, *Staph. caprae* is suspected of causing subclinical mastitis (Poutrel, 1984) and also of being responsible for human infections (Vandenesch *et al.* 1995). Types and occurrence of staphylococcal species in goat milk analysed in this work were similar to those reported by others (Harvey & Gilmour, 1988; Valle *et al.* 1990; Deinhofer & Pernthaner, 1995). However, the incidence of enterotoxigenic strains of *Staph. aureus* was lower, with a prevalence of strains producing enterotoxin C. Coagulase-negative staphylococci isolated did not synthesize known enterotoxins. Even though dairy products made from raw milk remain a potential danger, in the case of Caprino cheese, the acidification occurring during milk curdling (final pH in cheese paste $< 4{\cdot}5$) and the ripening carried out in refrigerated rooms (temperature < 10 °C) are conditions that are unlikely to support toxin production.

The authors thank Prof. G. Caserio, Prof. A. Galli (Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche of Milan) and Dr R. Lodi (Centro per lo Studio Tecnologico, Bromatologico e Microbiologico del Latte, CNR) for their scientific advice, and Dr P. Moroni (Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria of Milan) for his valuable collaboration. Many thanks are due to the farmers, F. Ardigò, M. Bonalumi, M. Costa, R. Facchetti, M. Gamba, C. Mapelli, G. Midali, L. Morali, G. Mosca and A. Vergani, for their interest, patience and help in collecting samples. This work was financed by a grant from MURST (CoFin 1997).

REFERENCES

Angelotti, R., Hall, H. E., Foter, M. J. & Lewis, K. M. 1962 Quantification of *Clostridium perfringens* in foods. *Applied Microbiology* **10** 193–199

Bielaszewska, M., Janda, J., Blahova, K., Minarikova, H., Jokova, E., Karmali, M. A., Laubova, J., Sikulova, J., Preston, M. A., Khakhria, R., Karch, H., Klazarova, H. & Nyc, O. 1997 Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. *Epidemiology and Infection* **119** 299–305

Camussi, A., Moller, F., Ottaviano, E. & Sari Gorla, M. 1986 [The Analysis of Variance] In [*Statistical methods for the biological investigation*] pp. 145–173. Bologna: Zanichelli Editore S.P.A

Chapman, G. H. 1945 The significance of sodium chloride in studies of Staphylococci. *Journal of Bacteriology* **50** 201–203

Coia, J. E. 1998 Clinical, microbiological and epidemiological aspects of *Escherichia coli* O157 infection. *FEMS Immunology and Medical Microbiology* **20** 1–9

Deinhofer, M. & Pernthaner, A. 1995 *Staphylococcus* spp. as mastitis-related pathogens in goat milk. *Veterinary Microbiology* **43** 161–166

DeMan, J. C., Rogosa, M. & Sharpe, M. E. 1960 A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology* **23** 130–135

Desmasures, N. & Gueguen, M. 1997 Monitoring the microbiology of high quality milk by monthly sampling over 2 years. *Journal of Dairy Research* **64** 271–280

Doyle, M. P. 1991 *Escherichia coli* O157:H7 and its significance in foods. *International Journal of Food Microbiology* **12** 289–302

Dumoulin, E. & Peretz, G. 1993 [Microbiological quality of raw goat milk in France]. *Lait* **73** 475–483

European Council Directive 92/46/EEC of 16 June 1992. Laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products

Foschino, R., Ottogalli, G. & Caserio, G. 1999 [Evolution of the microflora in the manufacture of traditional *Caprino* cheese]. *Scienza e Tecnica Lattiero-Casearia* **50** 1–16

Fraser, J. A. & Sperber, W. H. 1988 Rapid detection of Listeria spp. in food and environmental samples by esculin hydrolysis. *Journal of Food Protection* **51** 762–765

Frontini, S. 1997 [Goat milk and products]. *Latte* **22** 82–88

Gilmour, A. & Harvey, J. 1990 Staphylococci in milk and milk products. *Journal of Applied Bacteriology Symposium Supplement* **19** 147S–166S

Harvey, J. & Gilmour, A. 1988 Isolation and characterization of staphylococci from goats milk produced in Northern Ireland. *Letters in Applied Microbiology* **7** 79–82

International Dairy Federation 1990 *Milk and milk products. Yeasts and Moulds.* Brussels: IDF (*FIL–IDF Standard* no. 94B)

International Dairy Federation 1991 *Milk and milk products. Microorganisms. Colony count at 30* °C. Brussels: IDF (*FIL–IDF Standard* no. 100B)

International Dairy Federation 1992 *Milk and milk products. Preparation of samples and dilutions for microbiological examination.* Brussels: IDF (*FIL–IDF Standard* no. 122B)

International Dairy Federation 1994 *Enumeration of presumptive* Escherichia coli *content.* Brussels: IDF (*FIL–IDF Standard* no. 170)

International Dairy Federation 1995 *Milk and milk products. Detection of* Salmonella. Brussels: IDF (*FIL–IDF Standard* no. 93B)

International Dairy Federation 1997 *Milk and milk based products. Enumeration of coagulase-positive Staphylococci.* Brussels: IDF (*FIL–IDF Standard* no. 145A)

International Dairy Federation 1998 *Milk and milk products. Enumeration of coliforms.* Brussels: IDF (*FIL–IDF Standard* no. 73B)

Lodi, R. 1993 [Microbiological quality of raw goat milk in Italy]. *Lait* **73** 473–474

Lodi, R., Malaspina, P., Brasca, M., Magrini, M. & Bruni, G. 1994 [HACCP in goat cheese production]. *Latte* **19** 272–279

March, S. B. & Ratman, S. 1986 Sorbitol-MacConkey medium for the detection of *Escherichia coli* O157:H7 associated with haemorrhagic colitis. *Journal of Clinical Microbiology* **23** 869–872

Morgan, D., Newman, C. P., Hutchinson, D. N., Walker, A. M., Rowe, B. & Majid, F. 1993 Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. *Epidemiology and Infection* **111** 181–187

Mossel, D. A. A., Bijker, P. G. H. & Eeldering, J. 1978 [Streptococci of the D Group of Lancefield in foodstuffs and drinking water. Their significance, detection and control] *Archiv fur Lebensmittelhygiene* **29** 121–127

Mossel, D. A. A. & Van Netten, P. 1990 *Staphylococcus aureus* and related staphylococci in foods: ecology, proliferation, toxinogenesis, control and monitoring. *Journal of Applied Bacteriology Symposium Supplement* **19** 123S–145S

Poutrel, B. 1984 Udder infection of goats by coagulase negative staphylococci. *Veterinary Microbiology* **9** 131–137

Tarr, P. I., Besser, T. E., Hancock, D. D., Keene, W. E. & Goldoft, M. 1997 Verotoxigenic *Escherichia coli* infection: U.S. overview. *Journal of Food Protection* **60** 1466–1471

Terzaghi, B. E. & Sandine, W. E. 1975 Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology* **29** 807–813

Tirard-Collet, P., Zee, J. A., Carmichael, L. & Simard, R. E. 1991 A study of the microbiological quality of goat milk in Quebec. *Journal of Food Protection* **54** 263–266

Valle, J., Gomez-Lucia, E., Piriz, S., Goyache, J., Orden, J. A. & Vadillo, S. 1990 Enterotoxin production by staphylococci isolated from healthy goats. *Applied Environmental Microbiology* **56** 1323–1326

Vandenesch, F., Eykyn, S. J., Bes, M., Meugner, H., Fleurette, J. & Etienne, J. 1995 Identification and ribotypes of *Staphylococcus caprae* isolates isolated as human pathogens and from goat milk. *Journal of Clinical Microbiology* **33** 888–892

Vernozy-Rozand, C., Mazuy, C., Prevost, G., Lapeyre, C., Bes, M., Brun, Y. & Fleurette, J. 1996 Enterotoxin production by coagulase-negative staphylococci isolated from goats' milk and cheese. *International Journal of Food Microbiology* **30** 271–280

White, C. 1998 Testing milk and milk product. In *Applied Dairy Microbiology*, pp. 431–460 (Eds E. H., Marth & J. L Steele). New York: Marcel Dekker Inc

Wilson, D. J., Stewart, K. N. & Sears, P. M. 1995 Effect of stage of lactation, parity and season on somatic cell count in infected and uninfected dairy goats. *Small Ruminant Research* **16** 165–169

Zeng, S. S. & Escobar, E. N. 1995 Effect of parity and milk production on somatic cell count, standard plate count and composition of goat milk. *Small Ruminant Research* **17** 269–274

Zeng, S. S. & Escobar, E. N. 1996 Effect of breed and milking method on somatic cell count, standard plate count and composition of goat milk. *Small Ruminant Research* **19** 169–175