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Relationship between the Production Conditions of Goat's Milk and the Microbial Profiles of Milk

[1]H. Tormo, [2,3]C. Agabriel, [4]C. Lopez, [1]D. Ali Haimoud Lekhal and [5]C. Roques
[1]Université de Toulouse, Ecole d'Ingénieurs de Purpan, 75, voie du TOEC, BP 57611, F-31076 Toulouse Cedex 03, France
[2]Clermont Université, VetAgro Sup, UR UPSP DGER 2008.03.102, F-63012 Clermont-Fd Cedex 1, France
[3]INRA, USC 2005, F-63370, France
[4]Institut de l'Elevage, 149 rue de Bercy- 75595 Paris Cedex 12, France
[5]Université de Toulouse, UPS, LU 49, Adhésion bactérienne et formation de biofilms, 35 chemin des maraîchers, F-31062 Toulouse cedex 9, France

Corresponding Author: Tormo Hélène, Laboratoire de Microbiologie, Ecole d'Ingénieurs de Purpan, 75, voie du TOEC, BP 57611, F-31076 Toulouse Cedex 03, France Tel: +33 5 61 15 29 94 Fax: +33 5 61 15 30 60

ABSTRACT

The balance between microorganisms of technological interest and others in raw goat's milk have to be controlled to obtain good quality raw milk cheese. The aim of this study was: (1) to determine the microbial characteristics of goat's milk and (2) to evaluate factors that may have a potential influence (season, management practices). Thirty eight resulting farms were selected. A survey was carried out to qualify the management practices. A total of 228 milk samples were analyzed in spring 2006 and winter 2007. Microorganisms were counted in specific culture media. Statistical analysis was done to study the links between microbial composition of milks and management practices. The average Total Bacterial Count was 3.6 $\log_{10}$ cfu mL^{-1}. The major species were coagulase-negative staphylococci and, to a lesser extent, mesophilic acidifying bacteria, micrococci and corynebacteriaceae. Less than 60% of the milk samples were found to contain coagulase-positive staphylococci at very low levels. The levels of the main groups of microorganisms were significantly lower in Winter 2007. Microbial clusters of milk were established for the two seasons. These clusters differed in the levels of microorganisms, especially in the levels of mesophilic acidifying bacteria and micrococci and corynebacteriaceae. The relationship between management practices and microbial clusters of milk have underlined the importance of mixed practices depending on the season. The nature of the bedding and the temperature of the milking machine cleaning are the two mains factors closely linked to the microbial profile of milks. Present results underlined that it may be possible with a judicious choice of practices to increase the levels of mesophilic acidifying bacteria and micrococci and corynebacteriaceae, bacteria of technological relevance for the cheese making process, without altering the levels of undesirable bacteria (*Pseudomonas* spp., coagulase-positive staphylococci).

Key words: Milk, microorganisms, season, management practices, bedding, milking machine

INTRODUCTION

The microbial characteristics of milk are particularly important for controlling the hygienic and sensorial properties of raw milk cheese, as demonstrated by several studies (Demarigny *et al*., 1997;

Buchin and Beuvier, 2000; Verdier-Metz *et al*., 2005). For traditional raw milk cheeses, especially lactic acid goat's cheese, raw milk is a source of Lactic Acid Bacteria (LAB) in the whey. This whey, removed once the milk has coagulated, is used as a lactic starter (Tormo and Talliez, 2000). Other microorganisms of milk, such as yeasts or micrococci, also play a role in the ripening of soft cheese (Bhowmik and Marth, 1990; Bockelmann and Hoppe-Seyler, 2001). Nowadays, the sanitary quality of milk has been improved in France. But cheese farmers and scientists are beginning to wonder about the effect of the decrease in micro organisms of technological interest (Montel *et al*., 2003; Michel *et al*., 2005). Some studies on cow's milk have highlighted the relationships between the practices of cow's milk production and the levels of microorganisms or the balance between undesirable microorganisms and those of technological interest (Bouton *et al*., 2005; Michel *et al*., 2005). These practices concern the use of hay for food and bedding, the proximity between the forage and the cow shed and the cleaning practices used for the teats, the milking machine and the milking parlour. The few studies on goat's milk or goat's milk cheese have described groups of microorganisms for a small number of farms (Alonso-Calleja *et al*., 2002; Foschino *et al*., 2002; Callon *et al*., 2007). In addition, the management practices from the bedding to the milk production were not taken into account to explain the variability in the levels of microorganisms and especially, for different regions. The aim of this study is: (1) to investigate the variability in the microbial characteristics of goats' milk used for cheese manufacturing, sampled from 38 farms in two different regions: Protected Designation of Origin (PDO) Rocamadour and PDO Pelardon cheeses; (2) and then, to evaluate factors that have a potential influence: season, management practices such as bedding, milking conditions, cleaning and the maintenance of the milking machine and herd management.

MATERIALS AND METHODS

Study description: The present work was conducted in Spring 2006 and Winter 2007, in the Languedoc Roussillon and Midi-Pyrenees regions among producers of PDO farmhouse Pelardon (18 farms) and Rocamadour cheeses (20 farms), respectively. This study was carried out under real herd management conditions. For the study of breeding and milking production practices, the farms were selected by the Rocamadour and Pelardon cheese producers' trade unions. In order to have a wide variety of abiotic factors, two monitoring seasons were chosen:

- **Spring:** May-June 2006, labelled spring, as most flocks were outside in pasture (26 farms out of 38). The daily temperature was between 18 and 25°C, the lactation stage between 3 and 5 months for 70% or more of the goats in each flock. These factors could have an impact on the development and the nature of the microorganisms in the different environments (bedding, teats, milking machine, air around the bedding and milking parlour)
- **End of Winter:** February-March 2007 labelled "winter". All the flocks were housed, the daily temperature was between 10 and 18°C and the lactation stage inferior to one month for 70% or more of the goats in each flock

Data collection: For each farm, a survey was carried out for each sampling season. Some observations and measurements were included to qualify the management practices or the different environments. The observations and measurements were carried out by the investigators. Variables were categorized in seven families: (1) general characteristics of the farm (2) bedding management practices, (3) environmental conditions during and after milking, (4) cleaning practices and

cleanness of the milking machine, (5) milking machine maintenance and characteristics, (6) quality and cleaning of the teat and (7) cleaning practices of the dairy and milk tank. The different variables are described in Table 1. Some explanatory variables concerning (1) the cleanness of the milking machine (2) the bedding change (iii) the air entering the milking machine during milking, were reduced or transformed. The cleanness of 12 parts of the milking machine was recorded. When 2 or more parts were dirty, the cleanness of the milking machine was then noted as 'not clean'. In other cases, the milking machine was clean. The designation of the bedding change is the combination of the density and frequency of the bedding change (Table 1). The designation of the air entering the milking machine during milking is the combination between the number of units and the duration of the milking (Table 1).

Management practices were similar for each season. The results for the two seasons are presented in Table 1.

Sample collection: For each season, three milk samples were collected: on days 1, 4 and 8. The samples were collected in the tank from each farm from the evening milking. They were milking immediately cooled to 10°C and stored at -25°C for 15 days.

Counts on media and collection of isolates: The main groups of microorganisms which play a role in the sensorial and hygienic quality of milk were counted on culture media. Eleven media were selected (Table 2). A total of 114 samples (3 samples *38 farms) per season were inoculated onto each medium.

Each media was inoculated with 1 mL of pure or diluted sample (1/10 to $1/10^4$). Buffered peptone water (Biomérieux, France) was used for the dilutions. For the numeration of pseudomonas, yeasts and moulds, the samples were inoculated on the surface of the media. For the other microorganisms, the inoculation was carried out in the mass. The detection limit was set at 1 cfu mL^{-1} for inoculation of samples carried out in the mass. For micro-organism inoculated on the surface of the media, the detection limit was set at 10 cfu mL^{-1}.

Strain identification: We identified groups of bacteria or species using relatively unselective media (Blood sheep agar, Elliker modified by Chamba *et al.* (1981), Man Rogosa Sharpe +0.1% of vancomycin). A maximum of ten colonies with different morphotypes for each medium were selected.

- **Coagulase-Negative Staphylococci (CNS, cultivated on blood sheep agar):** after purification on Man Rogosa Sharpe agar, 100 strains were characterized using API staph (Biomerieux, France) after controlling morphology, gram staining and catalase activity
- **Mesophilic acidifying bacteria (MAB, cultivated on Elliker agar):** After purification over night, 177 strains were characterized. Phenotypic tests (gram, catalase, salt resistance, sugar fermentation) were performed and the strains with *Lactococcus lactis* or enterococci phenotypic profiles were selected. Strains were incubated at 30°C for 24 h in MRS broth and total DNA was extracted by using the Nucleospin tissue kit (Macherey Nagel, 67722 Hoerdt, France). The strains were confirmed to belong to *Lactococcus lactis lactis*, *lactococcus lactis cremoris*, Enterococci by means of PCR-based method. *Lactoccus actis* subsp *lactis* or subsp *cremoris* were identified using primers His 1 and His 2 (Corroler *et al.*, 1998). Enterococcal DNA were amplified using primers Conrev 23 and Genter according to Frahm *et al.* (1998). In all

Table 1: Groups of variables describing the management practices stable over the year and number of farms per practices

Variable level	Level	No. of farms
General management		
Size of the flock	= 132 goats	24
	> 132 goats	14
Pasture in spring and summer	yes	26
	no	12
Level of production (kg/goat/year)	= 650 kg	16
	>650 kg	22
Region	DOP Rocamadour	20
	DOP Pélardon	18
Lactation	all the year	16
	stop in winter	22
Management practices of bedding		
Bedding	straw	19
	Straw+hay	19
Bedding change[1]	unsufficient	5
	sufficient	17
	very sufficient	16
Additives in the bedding	yes	10
	no	28
Environmental conditions during and after the milking		
Air entering during the milking[2]	low : < 770	16
	medium [770, 1200]	10
	high = 1200	12
Cleanliness of the milking parlour	not clean: straw, hay and faeces	21
	Clean: no straw, hay and faeces	17
Frequency of the milking parlour cleaning	frequently: after each milking	27
	not frequently: less frequently than after each milking	11
Feed during the milking	no feed	11
	whole grains	19
	powder	8
Location of the milking parlour	no separation with the bedding area	14
	Physical separation	24
Cleaning and cleanliness of the milking machine		
Cleanliness of the milking machine	clean	24
	not very clean: some elements in the machine are not clean	14
Initial temperature of the milking machine cleaning	low: Ti = 55°C	13
	high: Ti>55°C	25
Final temperature of the milking machine cleaning	low: Tf = 40°C	20
	high: Tf>40°C	18
Air pipeline cleaning	very frequently: every month	12
	frequently: one to 4 times in a year	17
	unfrequently: less than one time in a year	9
Drying the pipeline of the milking machine	yes	24
	no	14
Water residue in the milking machine	yes	12
	no	26

Table 1: Continued

Variable level	Level	No. of farms
Maintenance and characteristic of the milking machine		
Age of the milking machine	< 10 years	19
	>10 years	19
Number of clusters	= 10	19
	> 10	19
Length of the pipeline	= 12 m	18
	> 12 m	20
Number of elbow and fittings	= 5	27
	>5	11
Changing of the liner	frequently: every year	18
	not frequently: less frequently than every year	20
Changing of the rubber pipes	Frequently: every two years	8
	not frequently than every two years	30
Status and cleaning of teats		
Status of teats	healthy teats	29
	teats with some indurations, ganglions, abcess...	9
Disinfecting teats after milking	yes	8
	no	30
Cleanliness of the dairy house and cleaning of the milk tank		
Cleanliness of the dairy house	clean	26
	not clean	12
Intercleaning with alkaline and acid products	frequently: change of product once or more in a week	23
	not frequently: change less than once in a week	15

[1]Bedding change insufficient: high density (<2.5 m^2/goat) and low frequency of change (less than 3 times in a year). Bedding change sufficient : low density (= 2.5 m^2/goat) and low frequency of change or high density and high frequency of change (more than 3 times in a year). Bedding change very sufficient : low density and high frequency of change. [2]Air entering during the milking : number of unit* 8l/min* duration of the milking

cases, amplification reactions were performed in a final volume of 12 µL contained 1X reaction PCR buffer (Qiagen, France), 0,3 µM of each opposing primers, 2,5 mM of $MgCl_2$, 0,2 mM of each deoxynucleoside triphosphate, 0,5 U Taq polymerase and 5 µL of DNA. The primer sequences and the PCR amplification conditions applied are recapitulated on Table 3. Amplifications were performed with a Thermal cycler (Biorad, 92430 Marne-la-coquette, France). PCR products were electrophoresed in a 8 g L^{-1} agarose gel (Sigma) in TBE at 100 V for 3 h. The 123-pb DNA ladder (Invitrogen, 95613 Cergy Pontoise, France) was used as a size standard. The DNA fragments were stained with ethidium bromide (sigma), viewed under UV light (302 nm) and photographed on a digital camera (G.Box, Syngene, UK)

- ***Leuconostoc* spp.:** These species have been cultivated in MRS (Man Rogosa Sharpe) agar with 0.1% of vancomycin). After purification overnight, 100 strains were identified using phenotypic tests. A lactate test was carried out on Gram positive, catalase negative rod strains using an enzymatic activity kit (Biosentec, France)

Data analysis: The number of microorganisms was expressed in $\log_{10}$ cfu mL^{-1} of milk.

Microbial characteristics of milk samples and variability for the two seasons: The description of the microbial characteristics of the milk samples was accomplished using descriptors

Table 2: Characteristics of the different culture media

Technological definition of microorganisms	Name of groups of microorganisms	Culture medium	Conditions of incubation (Temperature; time)	Colony count	Bibliographic references and suppliers
Total mesophilic bacteria	Total Bacteria Count (TBC)	Plate Count Agar+ 0.1% of skim milk powder	30°C; 72 h	All the colonies	IDF (1991a) DIFCO, France
Presumptive Lactic Acid Bacteria group	Mesophilic Acidifying Bacteria (MAB)	Elliker modified by Chamba *et al.* (1981)	20°C; 72 h	Yellow colonies	Chamba *et al.* (1981) BIORAD, France
Genus or species of Lactic Acid Bacteria	Enterococci Agar (BEA)	Bile Esculine Azide	37°C; 48 h	Colonies with black	AES, France
	Leuconostocs spp.	Man Rogosa and Sharpe+ 0.1% vancomycin	20°C; 96 h	All the colonies	AES, France
	Facultatively heterofermentative lactobacilli (LbII)	Isolini *et al.* (1990) +0.1% vancomycin	37°C; 48 h	All the colonies	Isolini *et al.* (1990)
	Micrococci and corynebacteriaceae (MCY)	Cheese Ripening Bacteria medium +0.1% furazolidone	20°C; 120 h	All the colonies	Denis *et al.* (2001)
Groups of microorganisms that are involved in cheese ripening	Coagulase-Negative Staphylococci (CNS)	Blood agar	37°C; 48 h	Grey and convex colonies	BIORAD, France
	Yeasts	Yeast Glucose Chloramphenicol agar (YGC)	20°C; 120 h	Colonies without convolution circles	IDF (1991b) AES, France
	Moulds	Yeast Glucose Chloramphenicol agar (YGC)	20°C; 120 h	Colonies with convolution circles	IDF (1991b) AES, France
Potentially pathogenic microorganisms	Coagulase-Positive Staphylococci (CPS)	Baid Parker+Rabbit Plasma Fibrinogen	37°C; 48 h	Dark colonies with halos	Beckers *et al.* (1984) Biomérieux, France
Microorganisms that Lactose A	Coliforms	Violet Red Bile Lactose A	30°C; 48 h	Red colonies with diameter> 0.5 mm	IDF (1985) AES, France
	Pseudomonas spp.	Cetrimid agar	20°C; 120 h	All the colonies	AES, France

Table 3: Primer sequences, amplification and application of PCR reactions

Primer sequences	Amplification conditions	Application
His1: 5'-CTTCGTTATGATTTTACA -3' His2: 5'- AATATCAACAATTCCATG-3'	5 min at 94°C, 30 cycles of: 1 min 94°C, 2 min at 45°C, 2 min at 72°C, final step 5 min at 72°C	Confirmation of the subspecies *Lactococcus lactis* lactis and cremoris
Conrev 23 : 5'- GGTGGATGCCTTGGCACT -3' Genter : 5'-CTCTACCTCCATCATTCT-3'	5 min at 94°C, 30 cycles of : 30 sec 94°C, 30 sec at 52°C, 30 sec at 72°C, final step at 4°C	Confirmation of the genus Enterococcus

for each group of microorganisms: mean, standard deviation, percentage of milk samples in which none of the different groups of microorganisms were detected (SAS 9.1, USA). Those variables were calculated for the 114 milk samples from each season.

The relationships between the microbial characteristics of the milk samples from 38 farms and the two seasons, were analyzed using an ANOVA mixed model (SAS 9.1, USA). The dependent variables were the levels of different microorganisms in the milk samples, the independent variables were the seasons (fixed effect in the model) and the farms (random effect in the model). The repeated measurements during the two seasons allowed us to estimate the random farm effect by means of a repeated option in a mixed SAS procedure. The variance between farms with regard to the total variance of the random effects (variances between farm and variance within the same farm) was estimated by the coefficient of correlation ñ. If the variance within a farm is high, the effect of the studied factor (season) is minimized.

Microbial clusters of milk: Principle Components Analysis was carried out on the Microbial Clusters of Milk for each season. The milk samples represented in the principal components were then classified by ascending hierarchical clustering using Ward's method (minimization of intra-class variance). The SPAD software version 5.5 (France) was used for this data analysis. The Total Bacteria Counts (TBC) was not included in the treatment. The groups of microorganisms taken into account in the treatment were detected in more than 40% of the milk samples. For spring, it concerned: MAB, *Enterococcus* spp., *Leuconostoc* spp., facultatively heterofermentative lactobacilli (Lb II), micrococci and corynebacteriaceae (MCY), yeasts, moulds, coliforms, Coagulase Negative Staphylococci (CNS), Coagulase Positive Staphylococci (CPS), *Pseudomonas* spp. and for winter it concerned: MAB, *Enterococcus* spp., *Leuconostoc* spp., LbII, MCY, CNS.

The others (including TBC) were considered as supplementary variables. This treatment allows us to study the relationships between microbial clusters and management practices.

Relationship between management practices and microbial clusters of milkfor each season: To associate management practices with microbial clusters of milk, each farm had to be associated with one microbial cluster. There were three milk analyses per farm per season. These milks could belong to different microbial clusters of milk. So, the dominant cluster of the three (the same cluster for 2 or 3 analyses) was considered as representative of the farm. If for some farms, there were three different microbial clusters, these farms were not taken into account in the analysis.

For each season, the relationships between the microbial cluster and the management practices were derived by means of a Classification Tree (XLStat 2008, France) using the CHAID method (Kass, 1980). The interest of this method was to organize the management practices into a hierarchy and then, to make combination of practices associated to microbial cluster. The farms described in the microbial clusters are successively split into sub groups discriminated on the variables of management practices. The segmentation is made by means of the Khi ² tests. The method takes a progressive approach as far as the cut in 2 sub groups is made on the most discriminating explanatory variable. Subdivision stops when the degrees of meaning in the Khi² tests are superior to a threshold fixed at 5%. This classification tree was performed for each season. For farms with the same tendency of microbial profile for both seasons, the relationships between management practices and the microbial composition of the milk was assessed using discriminatory factor analysis (XLStat 2008, France).

RESULTS

Microbial characteristics of milk samples

Strains identification: We confirmed that grey convex colonies growing on blood agar medium were Coagulase-Negative Staphylococci (CNS): 89 isolates out of 100 tested were CNS.

The dominant group of microorganisms growing on Elliker modified medium was Lactic Acid Bacteria (173 colonies out of 177), with mainly *L. lactis lactis* and enterococci (161 out of 173 colonies). The *L. lactis cremoris* subspecies seems to be specific to two farms only.

The main colonies growing on MRS agar (80%) possessed the Leuconostoc species phenotype (rods, Gram+, catalase -, production of D lactate).

Microbial characteristics of milk samples and variability according to the seasons: The microbial characteristics of the milk samples for the two seasons are illustrated in Table 4. Despite strong differences between the two seasons, the dominant and subdominant groups of micro-organism were the same. First, CNS (3,4 log cfu mL^{-1} in spring, 3,16 log cfu mL^{-1} in Winter) were detected of in the majority of samples (99% of samples for Spring and 87% of samples for Winter). Second, MAB and MCY were detected with high difference of level and percentage of detection according to the season. The levels of these two groups were about five to one hundred times lower than that of the CNS (Spring vs. Winter). The hierarchy of levels for lactic acid bacteria (subdominant group), was the same for the two seasons. In decreasing order : *Leuconostocs* spp., *Enteroccoccus* spp. and Lb II. *Lactococcus lactis* was not able to be counted because there was no specific medium. Nevertheless, this specie was dominant in some farms. For the other microorganisms (CPS, yeasts and moulds) the level was very low, especially in Winter (less than 10 cfu mL^{-1}). The level of coliforms was depended strongly on the season. In Spring, the level was almost equal to lactic acid bacteria. In Winter, coliforms were not detected in 87% of milks.

The ANOVA analysis (Table 4) showed that the levels of each group of microorganisms were significantly lower (three to ten times lower for the main groups and one hundred times lower for

Table 4: Microbial characteristics of milk samples (mean value and standard deviation were expressed in $\log_{10}$ cfu mL^{-1}) for Spring (n = 114) and winter (n = 114)

		Winter 2007				
	Spring 2006	% of milk		% of milk		
Label	Means ±σ[1]	with no detection[2]	Means±σ	with no detection[2]	ρ[3](%)	Ratio[4]
TBC	$3.79\pm 0.58_a$	0	$3.41\pm 1.00_b$	2	18	2
Presumptive LAB group						
MAB	$2.60\pm0.88_a$	0	$1.17\pm 0.88_b$	30	14	27
Enterococcus spp.	$1.71\pm0.80_a$	4	$0.77\pm 0.93_b$	54	21	9
Leuconostoc spp.	$2.19\pm0.76_a$	0	$1.13\pm 0.96_b$	47	31	11
Lb II	$1.44\pm0.83_a$	15	$0.91\pm 0.96_b$	56	46	
Ripening Flora		0				
MCY	$2.64\pm0.50_a$	1	$1.20\pm 1.43_b$	56	12	27
Yeasts	$1.41\pm0.88_a$	14	$0.43\pm 0.71_b$	66	8	9
Moulds	$0.81\pm0.69_a$	27	$0.26\pm 0.56_b$	81	23	4
CNS	3.30 ± 0.62	1	3.16 ± 1.41	13		
Undesirable' flora						
CPS	$1.00\pm0.97_a$	43	$0.46\pm 0.77_b$	72	0	3
Coliforms	$2.27\pm0.84_a$	4	$0.23\pm 0.63_b$	87	20	111
Pseudomonas spp.	$1.29\pm0.99_a$	30	$0.17\pm 0.67_b$	94	18	13

[1]σ: Standard deviation; [2]: Percentage of milks with no detection of the different groups of microorganisms, [3]ρ(%): Coefficient of correlation of the random factor effect (farms) [4]Ratio: Ratio of mean for each groups of microorganism (Spring vs Winter). No. with different letters (a, b) were significantly different (p-value<0.001)

coliforms, in cfu mL^{-1}) for winter than for Spring (p-value less than or equal to 0.01) except for CNS. This result was mainly due to the high percentage of groups of microorganisms in the milk samples that were not detected during the Winter (Table 4). Despite a season effect, the variability into farms was very high (coefficients of correlation were very low).

Relationship between management practices and microbial clusters of milk for the seasons

Microbial clusters of milk: For Spring, the typology analysis (described in 2.6.2) highlighted 4 Microbial clusters of milk. This classification explained 64% of the total variability in the microorganisms. The results of the typology are illustrated in Table 5. The levels of the two microbial clusters A and D were completely opposed as far as the main groups of microorganisms and in particular MAB, *Enterococcus* spp., *Leuconostoc* spp., LbII, MCY, yeasts and coliforms were concerned. The cluster D was an interesting cluster for cheese making because the balance and the level of microorganisms were in favour of microbial group of technology relevance (LAB, MCY, CNS). However, coliforms were detected with a level appreciably equal to MAB.

The levels of the main groups of microorganisms in the microbial cluster of milk labelled C were slightly lower than the mean level of all the samples (MAB, *Enterococcus* spp. CNS, CPS and coliforms) except for LbII which was higher.

The microbial cluster B was not different than all the samples, except for CPS which were ten times higher.

For Winter, the typology analysis highlighted 4 microbial clusters of milks. This classification explained 50% of the total inertia. The result of the microbial clusters of milks is illustrated in Table 6. Yeasts and moulds, coliforms, *Pseudomonas* spp., CPS have not been mentioned in the Table 6 because the percentage of detection was very low (6 to 34%) and there was no significant of level for this. The microbial clusters of milks E and F had the lowest levels as far as the main groups of microorganisms were concerned (ten-fold lower than the mean level of all the samples)

Table 5: Characteristics of microbial clusters of milk samples for Spring (mean value and standard deviation were expressed in log_{10} cfu mL^{-1})

	Microbial clusters of milk samples				
Characteristics	A	B	C	D	Total sample
No. of milks	22	31	34	27	114
TBC	**3.38± 0.29**	3.67±0.25	**3.58±0.32**	**4.52±0.63**	3.79±0.58
MAB	**1.76± 0.37**	2.61±0.47	**2.24±0.44**	**3.72±0.83**	2.60±0.58
Enterococcus spp.	**0.98±0.54**	1.88±0.48	**1.38±0.52**	**2.52±0.76**	1.71± 0.80
Leuconostoc spp.	**1.42±0.50**	2.01±0.43	2.11±0.42	**3.11±0.64**	2.19± 0.76
LbII	**0.48±0.62**	1.32±0.59	**1.75±0.57**	**1.97±0.78**	1.44±0.83
MCY	**2.22±0.67**	2.63±0.34	2.72±0.33	**2.87±0.46**	2.63±0.50
CNS	3.09±0.54	3.42±0.33	**2.88±0.66**	**3.85±0.41**	3.30±0.50
Yeasts	**0.91±0.90**	1.27±0.81	1.29±0.72	**2.13±0.65**	1.41±0.88
Moulds	0.6±0.54	0.93±0.69	0.74±0.63	0.93±0.79	0.81±0.69
CPS	1.33±0.92	**1.93±0.58**	**0.19±0.39**	**0.67±0.82**	1.00±0.97
Coliforms	**1.57±0.72**	2.43±0.52	**1.94±0.61**	**3.09±0.75**	2.27±0.84
Pseudomonas spp.,	1.05±0.89	1.37±1.01	1.15±0.97	1.58±0.97	1.29±0.99

The numbers in bold are the values significantly different to the total sample (p value<0.01). The points are the microorganisms not taken into account in the construction of clusters

Table 6: Characteristics of microbial clusters of milk samples for winter (mean value and standard deviation were expressed in $\log_{10}$ cfu mL^{-1})

Characteristics	E	F	G	H	Total sample
No. of milks	34	15	32	33	114
TBC	3.25±0.83	**2.60±0.66**	3.39±1.16	**3.97±0.75**	3.41± 0.99
MAB	**0.89±0.81**	**0.26±0.53**	1.30±0.63	**1.72±0.84**	1.17± 0.88
Enterococcus spp.	0.53±0.73	**0.31±0.53**	**0.09±0.28**	**1.88±0.61**	0.77±0.92
Leuconostoc spp.	**0.33±0.65**	**0.27±0.57**	**1.68±0.63**	**1.82±0.96**	1.131±0.96
LbII	**0.17±0.47**	**0.19±0.49**	**1.34±0.83**	**1.58±0.97**	0.91±0.97
MCY	**0.15±0.61**	0.72±1.52	**2.01±1.32**	**1.72±1.33**	1.20±1.43
CNS	3.42±0.67	**0.00±0.00**	**3.77±0.63**	**3.75±0.82**	3.16±1.41

The numbers in bold are the values significantly different to the total sample (p value<0.01). The points are the microorganisms which are not taken into account in the construction of clusters

Table 7: Number of farm for each microbial cluster associated with specific combination of management practices for Spring

	Microbial clusters of farms			
Combination of management practices	A (7[1])	B (11)	C (10)	D (7)
Bedding with straw with no additive in the bedding and final temperature of the milking machine cleaning >40°C and miliking parlour clean	3	0	0	0
Bedding with straw and hay and important change of bedding and milking parlour cleaning with dry method	4	0	0	0
Bedding with straw and hay and a low or medium frequency of change of bedding	0	0	0	7

[1]The numbers in parenthesis are the total numbers of farms for each microbial cluster of farms

excepted for CNS (no difference with the total sample for E and significantly lower for F). The microbial clusters of milks G and H had the highest levels of the majority of the microorganisms taken into account in the construction of the classification. The level of enterococci was 60 fold higher for H than for G. Those two microbial clusters could be considered rather as microbial profile of technological relevance for cheese making because the level of lactic acid bacteria (*Leuconostocs* spp., Lb II) and MCY was higher than the microbial clusters E and F.

Relationship between management practices and the microbial clusters of milk: For Spring, the three milk samples of three farms were classified in 3 different microbial clusters. These farms could not be analyzed, so 35 of the 38 farms were used for the analysis.

The Classification Tree has underlined some significant links between microbial clusters of milks and management practices. The microbial clusters of milks A, the least charged in microorganisms, was associated with 2 types of management practices (Table 7). The first type concerned farms using straw and hay for bedding with regular changes of bedding and a dry method of cleaning the milking platform. The second management practice included farms using only straw for bedding with no additives, a final milking machine cleaning temperature higher than 40°C and a clean milking parlour. Therefore, this microbial cluster was associated with management practices which reduce the levels of microorganisms. In contrast, the cluster D, the most charged in microorganisms concerned farms using straw and hay for bedding and changing the bedding less frequently than the cluster A. For the other clusters, no major profile of practices was registered.

Table 8: Number of farm for each microbial cluster associated with specific combination of management practices for Winter

	Microbial cluster of farm		
Combination of management practices	E(15[1])	G(8)	H(8)
Stop of milking in winter and final temperature of the milking machine cleaning >40°C	8	1	0
Final temperature of the milking machine cleaning = 40°C	4	1	8

[1]The numbers in parenthesis are the total numbers of farms for each microbial cluster of farms

Table 9: Percentage of farms in the two microbial cluster F- and F+ associated with management practices (Spring and Winter seasons)

	Farms affected to Microbial clusters (%)	
Management practices	F-	F+
Bedding with straw	**80.00**	0.00
Bedding with straw and hay	20.00	**100.00**
Initial temperature of the MM cleaning > 55°C	**100.0**	33.33
Initial temperature of the MM cleaning = 55°C	0.00	**66.67**

The numbers in bold underline the dominant practice significantly associated with microbial clusters (p value <0,05)

For the Winter, 31 of the 38 farms were used for the analysis. Five farms were not analyzed because the three milks were classified in 3 different microbial clusters and 2 farms were not analyzed as they were the only two that belonged to the microbial clusters of milks F. Consequently, this microbial cluster was removed from the analysis.

For eight farms on the fifteen in the E cluster (the least charged in microorganisms) the final milking machine cleaning temperature was higher than 40°C and they stopped the milk production in December and January (Table 8). For those farms, the final temperature higher was significantly associated with an initial milking machine cleaning temperature higher than 55°C and a daily change of the acid and alkaline milking machine cleaning product (Pearson's chi-square test, p-value<0.05).

In contrast, for the microbial cluster H (the most charged in microorganism), all the farms had a final temperature equal to or less than 40°C (Table 8). Those farms was significantly associated with an initial milking machine cleaning temperature below or equal to 55°C and changing the acid and alkaline milking machine cleaning product less than daily (Pearson's chi-square, p-value<0.05). For the cluster G, no major profile of practices was registered.

Relationship between the management practices and the microbial clusters of milks including the two seasons: Eleven of the 38 farms had similar microbial clusters of milk for the two seasons. Five farms had the lowest levels of the main groups of microorganisms and the three samples from each season were in the microbial cluster the least charged in microorganisms. Six farms had the highest levels of the main groups of microorganisms (labelled F+ for the two seasons) and were in clusters the least charged in microorganisms in Spring (cluster D) and in winter (cluster H).

The discriminatory factor analysis showed the significant effect of the management practices for the two groups of farms (Table 9), which concerned: the nature of the bedding and the initial and final temperature of milking machine cleaning. The practices linked to the microbial clusters of milks F- tended to limit the microorganisms in the environment. In contrast, the practices linked to the microbial clusters of milks F+ tended to increase the microorganisms in the environment.

DISCUSSION

The low TBC levels found in this study are similar to those found in studies on cow's milk (Michel *et al.*, 2001; Bouton *et al.*, 2005; Ercolini *et al.*, 2009). The dominance of CNS in goat's milk confirms previous findings (Valle *et al.*, 1991; Kyozaire *et al.*, 2005). The other major groups include bacteria of technological cheese relevance: MAB (mainly represented by the lactic acid bacteria) and MCY (cheese ripening bacteria). *L. lactis*, *Leuconostoc* spp. and *Enterococcus* spp., are the major lactic acid bacteria found, as underlined in a study on cow's milk by Zamfir *et al.* (2006) and on goat's milk by Badis *et al.* (2004). Concerning MCY, some studies reported their sub-dominance in goat milk (Tornadijo *et al.*, 1996; Garcia *et al.*, 2009; Callon *et al.*, 2007). The other groups of microorganisms (yeasts, moulds, *Pseudomonas* spp., CPS) were found in milk in very low levels (75% of milk samples <100 cfu mL^{-1}) as were the levels in cow's milk (Bouton *et al.*, 2005; Raynaud *et al.*, 2005; Michel *et al.*, 2005). The number of coliforms strongly depended on the season. In Spring, the levels were approximately equal to those of MAB and MCY; in winter the counts were one hundred times lower and they were only detected in 13% of the milk samples. Except for CNS, the numbers decreased in Winter, which is similar to the trends in cow's milk (Bouton *et al.*, 2005; Raynaud *et al.*, 2005; Michel *et al.*, 2005) and in milk from small ruminants in the Mediterranean area (Kalogriou-Vassiliadou *et al.*, 1991; Salmeron *et al.*, 2002). Yeasts and moulds were commonly found during the Spring in low levels in comparison with the other microflora. These levels are similar to those found in cow's milk (Michel *et al.*, 2001; Bouton *et al.*, 2005). During the Winter, only 20 to 30% of the milk samples contained yeasts and moulds. As far as we know, this variability of the presence of yeasts and moulds due to the season has never been reported in goat milk. The detection of *Pseudomonas* spp., in milk was also strongly affected by the season. The main factor which could explain this microbial variability is probably the fact that it is colder in Winter. The temperature may reduce the capacity of environmental microorganisms to multiply. In addition, the lactation stage may affect the microbial composition of milk. Some studies show the incidence of lactation on microorganisms involved in ruminant mastitis (Bergonnier *et al.*, 2003), but no data have been published for other microorganisms.

The analysis of the relationships between management practices and microbial clusters of milk has highlighted the effect of mixed practices on microorganisms. These practices depended on the season, but the trend was the same: there is a total contrast between the practices associated with milk samples containing low levels of microorganisms and milk samples containing high levels. Generally, practices that limit the level of microorganisms in the environment (bedding, milking machine) were associated with low levels of microorganisms in the milk samples, especially Lactic Acid Bacteria. In addition, the analysis of the relationships between management practices and microbial clusters of milk for farms with the same microbial profile for the seasons has highlighted the major effect of the nature of the bedding and the initial temperature of the milking machine cleaning. The level of the main groups of microorganisms in the milk sample was higher when the bedding was made from straw and hay rather than only straw. Present results show that for the same nature of bedding, the level of microorganisms for Spring is linked to the change of bedding or the final cleaning temperature. Concerning the bedding, our results support those by Sevi *et al.* (2003) and Albenzio *et al.* (2005), who showed that the maintenance of the bedding was important for the level of the microorganisms of cow's milk. Furthermore, our study underlined that the nature of the bedding could have an impact on the microbial composition of milk.

The milking machine cleaning temperature is highlighted for the two seasons. *Enterococcus* seems to be selected when the final milking machine cleaning temperature is lower than 40°C for Winter.

The effect of the cleaning conditions of the milking machine, often associated with global hygienic condition on the level of microorganisms was underlined for some studies in cow's milk (Chatelin and Richard, 1981; Michel *et al.*, 2001). In addition, Kaglki *et al.* (2006) found that the major clones of lactobacilli and enterococci isolated in a farm dairy environment come from the milking machine and the bulk-tank. So we may suppose that, when the temperature of the milking machine cleaning is lower, these bacteria could be improved, as it was shown in our study, especially for enterococci. In addition, farmers which stop goat lactation in winter and apply practices which decrease levels of microorganisms (final milking machine cleaning temperature higher than 40°C) have milk samples with very low levels of microorganisms. This result is probably due to the combination of the effects of the season (low temperature), the cleaning practices and the probable reduction of microorganisms in the milking machine during the drying off period for the goats. This report could explain the difficulty underlined by the farmer in making cheese in the beginning of the lactation. No studies have been reported this result to our knowledge.

CONCLUSION

The results indicate that there is a great variability in microbial composition of raw goat's milk except for CNS, which dominate. However, the microflora of technological interest in the cheese making process (Lactic acid bacteria and MCY) was detected as dominant in some milk samples. The low levels of CPS and *Pseudomonas* spp. (less than 200 cfu mL^{-1}) was proof of the good hygienic quality of the analysed milk samples. The variability in microbial composition was associated with (1) the season (2) management practices and (3) the interaction between season and management practices. These very low levels during the winter, especially for the farms that stop lactation, may explain the difficulties observed in making cheese at the beginning of the lactation season. The relationships between management practices and microbial clusters of milks have underlined the importance of mixed practices, including the nature of bedding, the cleanness and cleaning of the milking parlour and the milking machine. In addition, for farms which stop milk production, the absence of contact of the milk in the milking machine might reduce the level of microorganisms during the beginning of the lactation, especially lactic acid bacteria. For the first time in France, this study has highlighted the microbial composition in goat's milk from a high number of farms in two different regions and the relationships between the microbial profile and management practice itineraries using classification trees. The levels of LAB and in a lesser measure, MCY, may be increased by practices which transfer microorganisms from bedding to the milking machine. The above-mentioned practices seem to increase the levels of 'ambiguous' enterococci in milk and coliforms for the Spring season. So, it is necessary to more specify the environment and practices that favour microorganisms of interest in cheese technology such as *L. lactis* for example. Then, the relationship between these practices, the microbial profile of milk and the hygienic and sensorial quality of cheese could be investigated.

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