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Mastitis of dairy small ruminants

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Abstract – Staphylococci are the main aetiological agents of small ruminants intramammary infections (IMI), the more frequent isolates being *S. aureus* in clinical cases and coagulase negative species in subclinical IMI. The clinical IMI, whose annual incidence is usually lower than 5%, mainly occur at the beginning of machine milking and during the first third of lactation. These features constitute small ruminant peculiarities compared to dairy cattle. Small ruminant mastitis is generally a chronic and contagious infection: the primary sources are mammary and cutaneous carriages, and spreading mainly occurs during milking. Somatic cell counts (SCC) represent a valuable tool for prevalence assessment and screening, but predictive values are better in ewes than in goats. Prevention is most often based on milking machine management, sanitation and annual control, and milking technique optimisation. Elimination mainly relies on culling animals exhibiting clinical, chronic and recurrent IMI, and on drying-off intramammary antibiotherapy; this treatment allows a good efficacy and may be used selectively by targeting infected udders only. Heritability values for lactation mean SCC scores are between 0.11 and 0.15. Effective inclusion of ewe's mastitis resistance in the breeding goal has recently been implemented in France following experimental and large scale estimations of genetic parameters for SCC scores.

ewe / goat / mastitis / somatic cell count / epizootiology

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1. INTRODUCTION

Intramammary infections (IMI) in dairy small ruminants are mainly of bacterial origin; this paper does not deal with lentiviral or mycoplasmal infections (see [15, 19]). Ovine and caprine IMI epizootiology and control share numerous common points, but are different from some physiopathological points of view: in goats, there are shorter (or even no) dry period, more varied variation factors of somatic cell counts (SCC), lentiviral mammary infection, higher 'stress' susceptibility, etc. These also differ due to certain husbandry methods: in goats, generally lower kidding synchronisation, a

short or absent suckling period, diversity of alimentation systems from zero-grazing to traditional extensive husbandry, etc. Thus control strategies are based upon the same principles, but vary to a certain extent. On the contrary, important differences exist regarding cow IMI, which first led to improving basic knowledge and more recently to validating specific control programmes.

The importance of mastitis is mainly economic, hygienic (dairy products consumers) and legal in Europe (E.U. Directives 46/92 and 71/94 defining the bacteriological quality of milk).

2. AETIOLOGY

2.1. Clinical mastitis

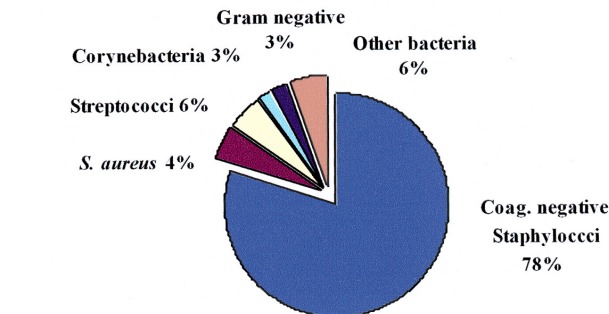
In sporadic cases for sheep and goats, the high prevalence of *Staphylococcus aureus* has often been reported. In a decreasing order of frequency, isolates belong to Coagulase Negative Staphylococci (CNS), which cannot be considered as minor pathogens in small ruminants, Streptococci, Enterobacteria, *Arcanobacterium pyogenes*, *Corynebacteria*, *Pasteurellaceae*, *Pseudomonas* spp., etc. [5, 14, 80, 146, 153].

Enzootic or epizootic outbreaks are due to *S. aureus*, then to *S. uberis*, *S. agalactiae*, *S. suis* (mainly during lactation) or to opportunistic pathogens such as *Aspergillus fumigatus* and *Pseudomonas aerugi-*

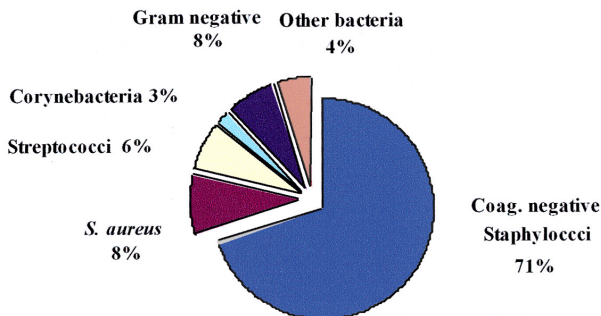
nosa (peri partum period and sometimes drying-off), and more rarely to *Burkholderia cepacia* or *Serratia marcescens* ([13, 23, 76, 110, 111, 145], Bergonier et al., unpublished results).

2.2. Subclinical mastitis

Figure 1 shows that CNS are the most prevalent, ranging from 25 to 93 %, before *S. aureus* (3 to 37 %) mainly isolated from infections that had become chronic (less severe ones). Among the CNS, *S. epidermidis* then *S. xylosus*, *S. chromogenes* and *S. simulans* are the more frequently isolated in ewes. In goats, *S. caprae* is one of the most prevalent species, followed by the former ones. In addition, among the CNS, *S. epidermidis* is generally associated with the highest average values of SCC both in



a : Aetiology of dairy ewe subclinical intramammary infections
(Mean data. See references in the text)



b : Aetiology of goat subclinical intramammary infections
(Mean data. See references in the text)

Figure 1. The aetiology of ovine and caprine subclinical intramammary infections.

ewes and goats, on the contrary to *S. caprae*. Sixty to more than 80% of CNS strains isolated from subclinical IMI produce evidence of alpha, delta or synergistic haemolysis. These haemolytic strains induce significantly higher SCC than non-haemolytic ones. Leucotoxin production is absent or lower in CNS than in *S. aureus*. In the latter species, caprine and ovine mastitis isolates are more leukotoxic than bovine ones. *Listeria monocytogenes* and *Salmonella* spp. IMI are very rare but important; these organisms can cause chronic and subclinical IMI [2, 8, 14, 17, 21, 28, 29, 38, 40, 68, 70, 75, 80, 109, 115, 129, 130, 143, 144].

3. DESCRIPTIVE EPIZOOTIOLOGY: RECENT KNOWLEDGE

3.1. Incidence, prevalence and persistence

3.1.1. Clinical mastitis

The incidence is usually lower than 5% per year. In a low percentage of herds, the incidence is higher and may exceed 30–50% of the animals, causing mortality or culling of up to 70% of the herd. *S. aureus*, Streptococci or opportunistic pathogens generally cause these outbreaks [32, 60, 69, 71].

The persistence of individual clinical IMI during lactation depends on the technical level, the flock size and the type of milk payment and valorisation (raw versus pasteurised milk). It is rarely documented and traditionally high, except for peracute cases [20]. Mastitic animals are not often immediately culled, and acute cases may become chronic for several months or more (1.5 to more than 30%). In ewe flocks, culling for IMI is increasing up to 7% of total causes in the Lacaune breed (Lagriffoul, unpublished results). In specialised goat herds, 18% of the animals culled or dead for disease reasons experienced mastitis. In the

two species, mammary pathology is the first cause of culling for sanitary reasons; it is more frequent during the first 2–3 months of lactation [92].

The persistence of mammary symptomatology after clinical cases during the dry period is not well documented but probably high [131], above all in goat husbandry characterised by a shorter dry period (1 to 3 months) than in that of the ewe (2 to 5 months). Culling of mastitic (even chronic) animals is highly recommended.

3.1.2. Subclinical mastitis

The prevalence may be assessed, as in dairy cattle, by the analysis of bulk milk somatic cell counts (bSCC), which is the only easy and cheap way to estimate the whole flock/herd mammary infectious status (exhaustive individual SCC [iSCC] are rarely available). In several regions, bSCC are carried out every month (or more) in every flock. Recent data are available regarding the relationship between bSCC annual mean or punctual values and the rate of presumed infected animals [73]. In ewes, the annual mean bSCC (weighted by the volumes) is closely linked ($r^2 = 0.845$) to the proportion of presumed 'persistently infected' ewes, using the thresholds proposed by Bergonier and Berthelot [14]. An increase of 100 000 cells per mL was associated with a rise of estimated prevalence of about 2.5% [73]. In Spanish flocks with 250 000 and 1 million cells per mL, the prevalence was estimated to 16 and 35% respectively, considering as infected an ewe with iSCC over 340 000 cells per mL [120]. The bSCC values and their correspondence with iSCC allow a good estimation of prevalence.

In goats (Fig. 2), this relationship is rather delicate to define, taking into account the incidence of lentiviral mastitis and the importance of non infectious variation factors of SCC. The influence of the kidding season on the bSCC values has been reported [52]. The dynamics of infections

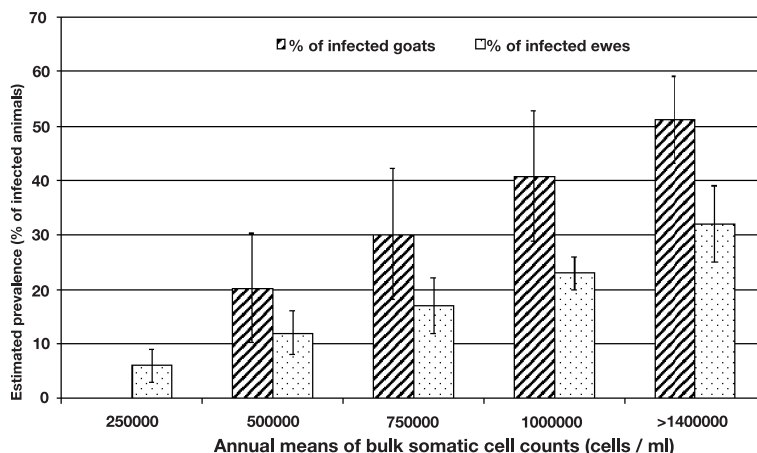


Figure 2. Relation between annual geometric mean of bulk somatic cell counts and prevalence of subclinical intramammary infections estimated by individual somatic cell counts in goats and ewe herds/flocks ([73, 74], De Cremoux, unpublished results).

can also vary according to the flock's structure and breeding management: percentages of primiparous goats or prolonged lactation goats, period and distribution of droppings, etc. [43]. Preliminary results are available from 155 French herds: annual geometric means of 750 000, 1 000 000 and 1 500 000 cells/mL corresponded respectively to 30% ($\pm 12\%$), 39% ($\pm 8\%$), 51% ($\pm 8\%$) of presumed infected goats [42, 43].

Table I presents the geometric means of bSCC performed in various European areas (European programme FAIR CT 95-0881: 'Strategies of in farm control of SCC in ewe's and goat's milk').

The persistence of subclinical IMI during lactation is variable according to the causative pathogen but is generally high, since Staphylococci represent the more frequent ones [132]. Subclinical IMI are in general poorly detected and not eliminated at least during lactation. In two monthly surveys concerning a total of 768 udderhalves during entire lactations (eight months) in six dairy flocks, 45 to 50% of infected halves exhibited a single IMI

caused by one pathogen with an average shedding duration of three to four months at least (± 2.5 months). Two or three successive and different infections also occurred frequently (Bergonier and Berthelot, unpublished data).

The persistence of subclinical IMI during the dry period is important to consider regarding the treatment strategies. An overall self-cure rate of 35 to 67% and of 20 to 60% of halves was estimated respectively in the ewe [17, 51, 64] and goat [81, 98, 112, 114]. Thus, percentages of spontaneous cure during the dry period are generally lower in goats. If the infection substitutions during the dry period are taken into account, these percentages are lower.

3.2. Effects of stage and number of lactation on the IMI incidence and prevalence

3.2.1. Lactation stage

The incidence of clinical IMI does not vary with the lactation stage in the same way as in dairy cattle. A high incidence at

Table I. Geometric means of bulk milk somatic cell counts in various areas of the ewe's and goat's milk production (in thousands cells per mL) (Source: annual reports of the European programme FAIR CT 95-0881: 'Strategies of in farm control of Somatic Cell Counts in ewe's and goat's milk').

Species	Areas of production (breed)	1995	1996	1997	1998	1999	2000	Global
Ewes	Castilla-León (Churra)	–	–	783	866	916	–	863
	Castilla-La Mancha (Manchega)	758	660	776	741	602	691	708
	Spanish Basque country (Latxa)	–	481	482	473	–	–	477
	Roquefort (Lacaune)	713	599	680	657	647	586	647
	Pyrenees (Basco-béarnaise, Manech)	642	642	663	733	750	710	690
	Sardinia (Sarda)	1561	1624	1566	1416	1457	1493	1509
Goats	Castilla-La Mancha	2187	1259	1380	1318	1023	1071	1148
	France (Alpine, Saanen)	1218	1111	1166	1226	1252	1309	1214
	Sardinia (Sarda)	1595	1739	1516	1468	1483	1673	1575

drying-off or at parturition is observed in very rare and specific cases (mycotic agents or *P. aeruginosa*), in relation with environmental contamination and/or poor hygiene practices. On the contrary, the higher rates are observed at the beginning of machine milking and during the first third of lactation. High incidence may be observed in dairy ewes during suckling or suckling-milking periods [22, 76, 80].

The variations of subclinical IMI incidence according to the stage of lactation should be assessed by systematic monthly milk culturing of large numbers of healthy udders. This kind of a study is very rare. Variations can be indirectly estimated through SCC, allowing the analysis of large data sets. They must be cautiously interpreted as infectious and milk dilution/concentration effects are added. In goats, higher rates have been observed at the beginning of lactation [48, 128], but prevalence seems to increase throughout the campaign (Fig. 3). In ewes, a high incidence was reported at the beginning of lactation [70, 80].

3.2.2. Lactation number (parity)

An increased prevalence related to parity has been reported in ewes and goats [26, 56, 80, 138]. Such descriptive data must be cautiously interpreted: the incidence is difficult to differentiate from prevalence (chronicity or relapse), and older animals may be the more resistant ones.

4. ANALYTICAL EPIZOOTIOLOGY

4.1. Sources and associated factors

4.1.1. Sources

*The primary sources are firstly animal carriage and subclinical IMI. The main significant Staphylococci reservoirs are subclinical and chronic IMI and teat cutaneous infections (traumas, contagious ecthyma). CNS and *S. aureus* can also be cultured from healthy teat skin (and other body sites or external orifices) ([27, 135],*

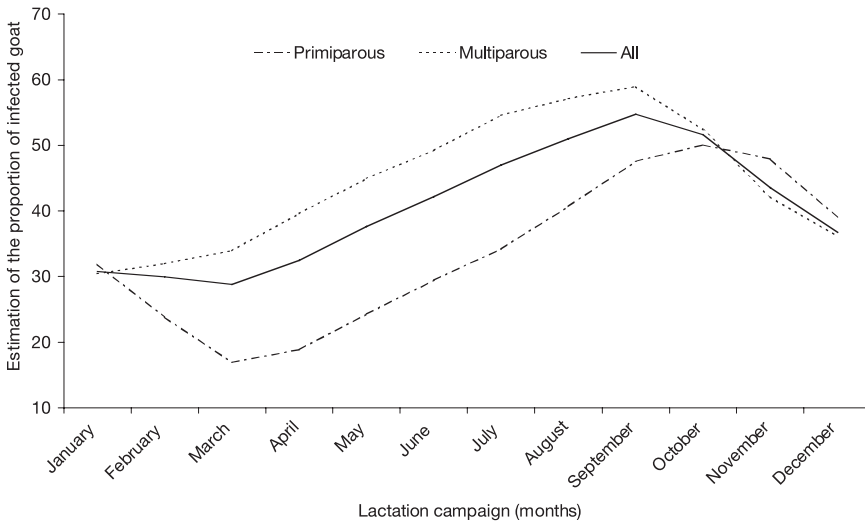


Figure 3. Estimation of intramammary infection average prevalence throughout lactation in 155 French caprine herds [43]. Parturition took place in November and December. (Proportions of presumed infected udders were estimated through individual somatic cell counts.)

Bergonier et al., unpublished results). Other bacteria also have animal primary sources: *S. agalactiae*, *A. pyogenes*, *Mannheimia haemolytica*, etc. The latter is carried in the adults and suckling young's mouth, nasopharynx and tonsils [134].

Other primary sources are environmental: Enterobacteria and Enterococci are found particularly in the litter, and *Pseudomonas* spp. especially in water or a humid environment. *A. fumigatus* and other fungi are isolated from mouldy forage, wet bedding, litter, and air [110]. *S. uberis* and *S. suis* recognise mixed reservoirs: infected animals, litter, and the environment.

The accessory sources are, for Staphylococci, housing, bedding, feedstuffs, air, insects, clusters, equipments, humans (hands), other animals, etc. [3, 28, 139]. *M. haemolytica* can be found on the teat skin of ewes soon after lambing [134] and in the environment of diseased animals: grass, water, straw bedding, etc. Colder, wetter weather seems to prolong the sur-

vival of this organism [27, 153]. *P. aeruginosa*, *S. marcescens* can survive in teat dipping solutions or disinfected moist clusters (Bergonier et al., unpublished results).

4.1.2. Factors associated to bacterial persistence

Udder infection persistence is due to the lack of precocious IMI detection and systematic application of control programmes: teat antiseptics, antibiotherapy or culling. In French dairy ewe recorded flocks (379 flocks of the Roquefort area), foremilk inspection is exceptional, udder palpation and California Mastitis Test (CMT) are occasionally realised by 57 and 65% of the farmers; post-milking teat antiseptics and drying-off antibiotherapy are performed in 20 and 70% of the flocks, respectively (Lagriffoul, 2000, unpublished results). These percentages are high compared to other dairy ewe areas and are increasing. In French goat herds, drying-off antibiotherapy frequency increased from 62.7%

in 1997 to 84.3% in 2000; teat antiseptics progressed from 13.7 to 29.4% of the herds [44].

Extra-mammary bacterial persistence is firstly due to hygienic or technical failures concerning the milking machine (over-used liners, etc.). Very little literature is available; incorrect machine disinfection (no alternation acid-alkaline chlorinated water, too low water temperature) or the use of rubber (vs. silicone) liners seems to be associated with poorer tank milk bacterial counts in ewes.

Secondly, high stocking density, particularly in intensively managed herds/flocks or during the suckling period, may result in large air concentrations of total microorganisms, mesophilic or coliform bacteria and staphylococci. These effects are probably associated with incorrect ventilation and high relative humidity. The multiplication of various bacteria on the skin (and in the litter) can be subsequently enhanced [2, 137, 139].

4.2. Factors of susceptibility

4.2.1. Genetic factors: recent advances

Several studies on resistance to mastitis have been recently based on SCC as an indirect way of measuring udder sanitary status in dairy sheep (no literature data is available in dairy goats).

Genetic parameters for SCC are calculated after logarithmic transformation of the values, i.e. somatic cell scores (SCS). The results based on repeatability test day models for SCS indicate variable heritability estimates from 0.04 to 0.17 [9, 10, 49, 63, 83]. Most studies based on larger data sets for the Churra and Lacaune breeds reported consistent heritability values between 0.11 and 0.15 for the lactation mean SCS [9, 50, 123, 124]. A high genetic correlation between the first and second lactation (0.88–0.93) has been reported, indicating that mostly the same genes deter-

mine SCS across lactation [123, 124]. The results are in close agreement with dairy cattle literature [125]. The genetic variability of SCS in dairy ewes appears sufficient to implement a selection. The evolution of the genetic determinism of SCS during lactation shows a moderate to strong increase in heritability. The values are particularly low on the first test days (0.01 to 0.04), then between the beginning to the middle of the lactation, estimates range from 0.04–0.1 to 0.12–0.25 [9, 10, 83]. In dairy cattle, comparable studies reported a smaller increase with days in milk, with higher values in the first months of lactation [33, 36, 118].

Regarding relationships between udder health and milk production traits, several studies indicate unfavourable positive genetic correlations between SCS and production traits, ranging from 0.1 to 0.2 [9, 96, 124], as in dairy cattle [125]. Additionally, the risk of being predicted as subclinically infected (according to French SCC thresholds) is significantly increased in the high, versus low, divergent lines selected on milk production in the La Fage experimental flock [9]. On the contrary, some authors found a favourable negative genetic correlation between SCS and milk yield, ranging from –0.35 to –0.11 [10, 49, 50, 83], concluding that selection for increased milk yield might not result in an unfavourable response for SCC. Discrepancies between the results could be due to differences in breeds, modelling and nature of the SCC data.

4.2.2. Environmental factors

Milking equipment and milking routine are the main receptivity factors during lactation. In ewes, association between teat injuries and mastitis has been reported [5]. The limitation of udder massages and stripping is associated with a significant positive effect for primiparous goats: a decrease of IMI and teat congestions. Over-milking limitation (respectively suppression) may be associated with the reduction of hyperkeratoses (respectively reduction of minor

pathogen IMI prevalence), but data concerning over-milking are rather inconsistent; it is probably better tolerated by healthy udders [44]. A vacuum level increase may induce a rise of SCC without clinical IMI [88, 140].

Intramammary infusions at drying-off or during lactation may cause teat duct cell damages.

The factors affecting sensitivity are not very well known (very few data are available about udder immunity). In dairy ewes, parenteral administration of vitamin E and selenium during the dry period can reduce SCC and increase the percentages of milk neutrophils during the subsequent lactation [102, 121]. Sensitivity may also be increased by milk retention, due to a bad working milking machine, under-milking, stressful or painful milking and even udder morphology. Milk kinetics analysis allows to evidence too short milking times: 12 to 14% of under-milking (1023 kinetics) have been observed in goats [24].

4.3. Transmission

Spreading mainly occurs during milking. Udder massage and stripping induce air intakes leading to impact. Cluster removal by the milker may also induce impact, since it is often performed without previous vacuum cutting off (the automatic cluster removal is developing). Bacteria are also transported passively by liners. However, the IMI prevalences do not seem to be significantly different between dairy (hand or machine milked) and meat flocks. Transmission is also possible by "milk-robber" lambs (buccal carriage) and may be important for staphylococci, *Pasteurellaceae*, parapox virus (contagious ecthyma), etc.

Penetration of the udder by organisms is performed via the teat duct. In cases of systemic infections, haematogenous colonisation is frequent (lentivirus, mycoplasmosis, brucellosis, etc.).

5. DIAGNOSIS: NEW DEVELOPMENTS IN THE CYTOLOGICAL DETECTION OF MASTITIS

Clinical and bacteriological diagnosis will not be considered, as recent advances mainly concern detection of subclinical IMI by SCC.

5.1. Milk cell subpopulations

Small ruminant milk contains the same types of cells as cow's milk, but important differences exist between ewe and goat milk cells in subpopulation percentages and total counts (Tab. II).

In bacteriologically negative *ewe's milk*, epithelial cells seem to be less than 2–3% of somatic cells, polymorphonuclear neutrophil leukocytes (PMNL) constitute 10–35%, macrophages 45–85% and lymphocytes 10–17%. These percentages do not vary to a large extent with the lactation stage, excluding the beginning of lactation (particularly the colostral period) and the involutive period: in early involution secretion, PMNL increase; they decline 21 days after cessation of lactation [78]. These percentages are close to those of cow's milk (2–30% for PMNL for example); in both species, the macrophage is the major cell type in bacteriologically negative milk [99, 106, 108]. There is a high correlation in ewes and cows between PMNL percentages and SCC [59, 103].

On the contrary, in goats, several cytological peculiarities must be underlined for their biological as well as operational (IMI detection) interests. First of all, milk secretion in goats is apocrine [149, 150]: cytoplasmic particles are physiologically shed into milk from the apical portion of secretory cells. Although most of them are anucleated, some of these particles have been observed to contain nuclear fragments [107] and could contribute to a slight extent to the total cell count. Cytoplasmic particles are similar in size to milk somatic cells.

Table II. Distribution of leucocyte subpopulations in small ruminant milk.

Species	Reference	Year	<i>n</i>	Milk type	Lactation (L.) stage	Neutrophils %	Macrophages %	Lymphocytes %	
Goats	[46]	1982	56	uninfected halves	early L.	45	35	20	
			4	uninfected halves	late L.	74	15	9	
	[45]	1993	70	bulk tank		87.3	9.9	2.8	
	[122]	1993	200	halves	weeks 1–4 pp	45.8 to 52.5	19.6 to 27.2	12.9 to 14.1	
				halves	weeks 28–31 pp	68.6 to 70.3	12.0 to 12.5	2.9 to 4.3	
	[93]	1998	12	halves	weeks 2–3 pp	–	80	–	
					weeks 4–18	50	45	–	
					≥ 19 weeks pp	80	–	–	
	[52]	1999	950	halves	–	40.9	35.6	0.7	
	[151]	2002	237	halves	early L.	79	–	22	
			36	halves	late L.	78	–	22	
Ewes	[55]	1985	84	uninfected halves	mid-L.	26.5 to 58.5	–	–	
			12	uninfected halves	early drying-off	69.7 to 85.8	–	–	
	[59]	1985	91	halves	mid-L.	10 to 90	0 to 60	8 to 18	
	[77]	1976	6	halves	dry udder	–	84	6	
	[78]	1981	6	halves	colostrum	41 to 84	8 to 49	6 to 11	
				6	halves	mid-L.	–	83 to 86	10 to 17
	[99]	1994	–	uninfected halves	whole L.	30	60	8	
	[100]	1996	640	uninfected halves	whole L.	34.9	–	–	
				50	infected halves	whole L.	52.1 to 82.2	–	–
	[101]	1996	10	healthy halves	whole L.	30.6	57.3	8.2	
[103]	2001	40	uninfected halves	mid and late L.*	31.1 to 52.6	–	–		
			infected halves	mid and late L.*	65.9 to 77.6	–	–		

pp: postpartum. * No significant evolution according to the lactation stage.

Their average concentrations in milk is $150 \times 10^3/\text{mL}$ for goats and $15 \times 10^3/\text{mL}$ for ewes [108]. Furthermore, numerous investigators reported unexpected SCC increases and high PMNL percentages (Tab. II): PMNL comprise the major cell type in milk from uninfected goats [108]. In normal late lactation milk, they constitute 74–80% of total cells. A significant increase has been found in PMNL chemotactic activity during this stage compared to early lactation and inverted evolution of mononuclear cells chemotactic activity. Differences between chemotactic cytokine profiles of mastitic and normal late-lactation-stage milk suggest the existence of a physiologic regulation in the influx of PMNL into the milk. The authors suspected PMNL to serve as physiological regulators in the early phase of the involution process [93]. In early lactation also, goat milk generally contains a higher PMNL proportion than the others. It has been suggested that leukocyte migration could proceed at a faster rate than that into cow's milk and might contribute to naturally higher SCC [107]. The hypothesis of different defense mechanisms for the goat udder than for the bovine udder has been considered [97]. Some authors suggest that in goats compared to dairy cattle, the low incidence of clinical IMI might be related to the physiologically high SCC and PMNL percentage. This hypothesis is weakened by the comparison with the ewes situation: the same low clinical IMI incidence and, on the contrary to goats, the same range of somatic cell counts and percentages as in the cow.

5.2. Lentiviral variation factors of somatic cell counts

The mammary gland of the goat and ewe is a target organ for lentiviral infections, but an additional difference between the two species is reported. *In the ewe*, no clear evidence is available for influence of Maedi-Visna infection on SCC at a large scale [79].

In goats, there is a little more information concerning the relationship between CAEV and SCC or the prevalence of sub-clinical IMI. A relationship between serological status and bacterial IMI was reported in herds with a high prevalence of CAEV and IMI [62, 127, 129, 142]. This was attributed to a selective immunosuppression due to altered macrophage function during CAEV infection. In seropositive goats, an increase of SCC was mostly reported as far as bacteriologically uninfected halves are concerned [82, 105, 127, 130]. This could be related to the larger number of macrophages in the milk of CAEV contaminated goats [82]. Nevertheless, it was weak [82, 129] and lower than those due to bacterial infections. The effect of CAEV on SCC was not apparent when udder halves showed a persistent subclinical IMI. This failure of the inflammatory response was interpreted as a reduction in the activity of macrophages caused by the viral infection. Some cytokines, such as chemotactic factors for neutrophils, and some mediators such as interleukin-8 could be involved in this process [129].

5.3. Non-pathological variation factors of somatic cell counts

The main variation factors for healthy udders are, in decreasing order of frequency, lactation stage, lactation number or within-day fluctuations, and milk fractions. The nature and importance of these factors have already been described [18, 60, 72, 108]. Briefly, in the ewe they are responsible for geometric mean variations ranging from 40 000 to 100 000 cells/mL, the mean iSCC values for uninfected ewe udders in mid-lactation ranging from 100 000 to 250 000 cells/mL [18, 58, 108, 123]. The lactation stage is the first of these factors. Irrespectively of the infectious status, SCC values increase as the stage of lactation progresses, above all in goats (Figs. 4 and 5): average values during the first three months are 200×10^3 cells/mL, and progressively

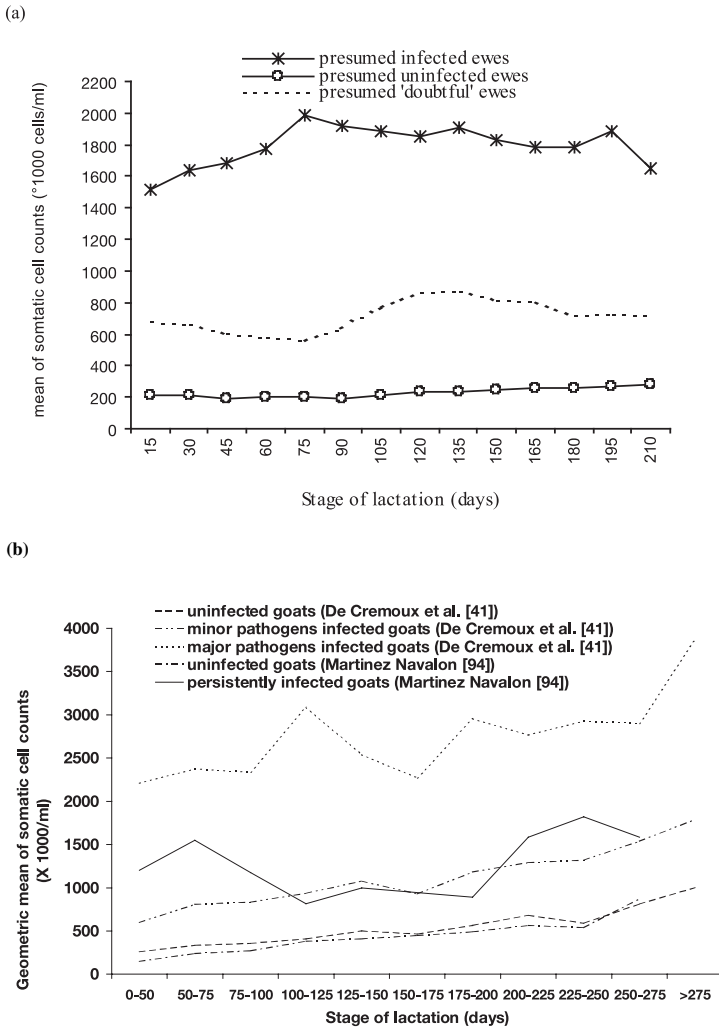


Figure 4. The effect of lactation stage on individual somatic cell counts according to the infectious status of the mammary gland of ewes (a) (Rupp, unpublished results) and goats (b) [41, 94].

increase to more than 10^6 cells/mL during the latter months [108]. In ewes, counts are higher during the first few weeks of lactation and decrease at the maximum milk production. Average SCC (and PMNL percentages) are also affected by parity (Fig. 5) [38, 41, 71, 122, 147]. For healthy ewe

udders, whose SCC values show less fluctuations than those of goats, the ‘flock-campaign’ effect appears to be the most important [123].

Minor or punctual SCC variation factors may exist according to the management of the flock/herd. In ewes, the number

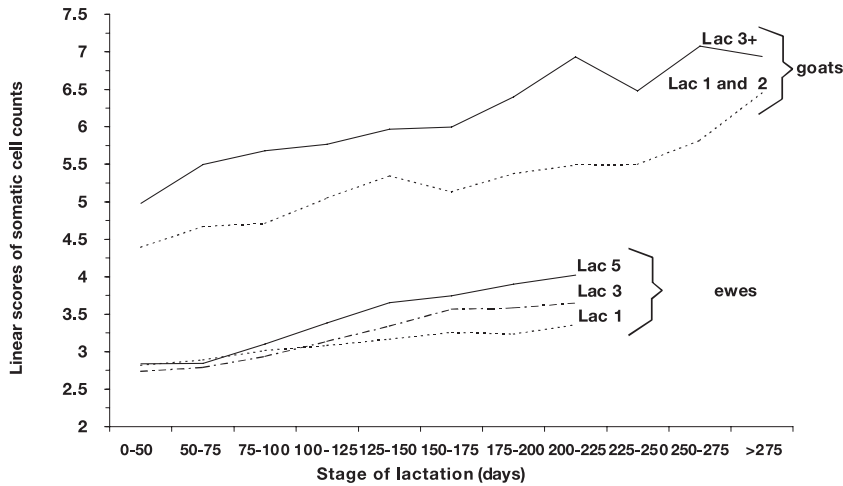


Figure 5. Illustration of the lactation number (parity) effect on individual somatic cell counts throughout the lactation of uninfected ewes [123] and goats [41]. Lac: lactation.

of suckling lambs, the suckling-milking period management, the lambing month, and sudden dietary transitions are responsible for mild variations, whose geometric mean is generally lower than 20 000 cells/mL [71, 72, 123]. In goats, vaccinations, treatments, abrupt dietary modifications, stress, etc. have been found to increase SCC [82]. A significant SCC increase with a simultaneous decrease of production has been observed during induced estrus. This effect is accentuated in infected halves ([4, 91], Poutrel, unpublished data).

In conclusion, important differences concerning differential and total counts, lentiviral infection effect and non-pathological variation factors exist between dairy ewes and cows and, on the other hand, goats. For the latter, cellular recruitment is characterised by a more important number and diversity of factors, and by greater inter-individual and temporal variations. The signification of goat milk cellularity fluctuations is not so far completely understood. Nevertheless, bacterial IMI is the main variation factor of SCC, which was found to be the best predictor of infec-

tion status among the indirect tests available at the moment [14, 91].

5.4. Recent advances in the practical use of individual somatic cell counts

5.4.1. Punctual approach (and single threshold)

This simple and quite early methodology proposes the punctual or instantaneous discrimination between 'healthy' and 'infected' udders, generally using a single threshold. Thresholds are sometimes proposed for milk to be tested bacteriologically. The threshold determination is usually based on the comparison of iSCC of punctually infected and uninfected halves, or on the choice of the best compromise between sensitivity and specificity. Briefly, an analysis of the available literature bring forth the differences on the technical and methodological levels [40]. In goats, the apocrine character of lacteal secretion implies specific coloration of DNA in order to differentiate cytoplasmic

particles from leukocytes [46, 152]. Large differences concerning the thresholds are also notable, even when considering studies using the fluoro-opto-electronic (FOE) method. The validity parameters (sensitivity, specificity, predictive values, etc.) of such detection methods are also highly variable and consequently will not be reported.

In ewes with the FOE method, the single thresholds proposed surprisingly range from 200 000 to 1.5 million cells/mL; nevertheless the majority of them are below 500 000 cells/mL [57, 58, 90, 95, 109, 119]. Some authors suggested using two thresholds to distinguish 'healthy' from 'infected' udders (140 000 and 340 000 cells/mL, [120]) or 'minor' from 'major' pathogen IMI (244 000 and 10^6 cells/mL, [144]).

In goats, for healthy halves, arithmetic means with the FOE method range from 520 000 to 1.1×10^6 cells/mL [37, 41, 82, 113] and geometric means from 223 000 to 396 000 cells/mL [37, 41, 90]. The cut-off values range from 500 000 to 1.5×10^6 cells/mL [37, 67]. The studies and results differ depending on whether they consider the nature ('minor' versus 'major' pathogens), intensity or persistence of IMI, and impact of non-infectious factors. Among them, lactation stage has been proposed to be included in the definition of a series of threshold values: according to certain authors, defining punctual standards for goat SCC is not possible without considering lactation stage and probably parity [60]. Using monthly SCC, a linear regression allowed the determination of cut-off values from 556 000 cells/mL (90 days in milk) to 1.2×10^6 cells/mL (305 days) [60].

5.4.2. Dynamical approach and multiple thresholds

In addition to the necessity to take into account these identified non-infectious variation factors, other reasons explain that studies have recently been focused on the

dynamic character of infection and cellular response. Cut-off recommendations have moved towards the use of consecutive iSCC per lactation and the definition of two (or three) thresholds within the same detection systems. An important reason is that staphylococcal IMI are characterised by dynamic fluctuations and consequently cyclic milk shedding, potentially causing false-negative bacteriological results. 'Intermittent isolations' have been reported [27, 132]. This bacterial cyclicality is generally inverted to PMNL one, since neutrophil infiltrates usually eliminate most but not all bacteria; SCC fluctuate according to the organisms number and viability [39, 116, 136, 148]. On the contrary, discordance between some chronic IMI and SCC values is due to the important heterogeneity in CNS field strain virulence. For example in ewes, the SCC mean induced by *S. lentus* is very close to that of uninfected halves [14].

Consequently, one should prefer to use detection systems defining a third class of 'doubtful' udders and the combination of several successive SCC, as in dairy cattle, rather than punctual approaches. A geometric mean is sometimes employed, but generally one or several criterions based on the number of SCC exceeding the thresholds are used. Methodologically, these proposed standards originate from the comparison of monthly SCC throughout the lactation to the results of monthly bacteriological analysis of udder-halve milk samples. In goats, since 2000, development of udder health management programmes in France is based on a synthesis of two studies [11, 41] (Tab. III) (SCC standards are used for milk quality payment). The latter defined thresholds related to various operational strategies, since the development of a single criterion is difficult in goats. Four criterions are proposed depending on the nature of the infections, period of detection (early lactation, drying off) and practices (preventive or curative) for controlling IMI. In ewes (Tab. III), it is also possible to use geometric means or numbers

Table III. Dynamic approaches for somatic cell count detection of subclinical mastitis.

Reference	Year	Infection type	Criterion	SCC N°	Threshold	Test validity parameters (%)				
						Sens.	Spe.	PPV	NPV	Eff.
Detection of infected goat halves										
[128]	1998	All infections	Geometric mean	6	1 100	57.4	75.9	15.6	95.8	74.7
[65]	1990	All infections	No. of over counting: 2		800	62.5	87.0	61.4	92.5	80.9
Detection of infected goat udders										
[41]	1995	Minor pathogens	No. of over counting: 2	6	750	82.6	60.9	77.5	68.2	73.1
		Major pathogens	No. of over counting: 3	6	1 750	61.3	80.2	16.5	97.0	79.2
[11]	1998	<i>S. aureus</i>	No. of over counting: 1	C1 + C2	3 000	81.8	95.3	42.9	99.2	94.7
		<i>S. aureus</i>	No. of over counting: 3	C3 to Cn	2 000	73.3	91.2	33.3	98.3	90.2
		<i>S. aureus</i>	No. of over counting: 2	C1 to Cn	2 000	100.0	74.1	18.7	100.0	75.6
		All (primiparous)	No. of over counting: 2	C1 to Cn	600	90.9	85.7	90.9	85.7	88.9
Detection of infected ewe udders										
[16]	1996a	Short infection or 'doubtful'	No. of over counting: 3	7	500	84.1	66.3	-	-	71.1
[14]	2003									

Sens.: sensitivity; Spe.: specificity; PPV and NPV: positive and negative predictive values; Eff.: efficiency.

Thresholds: × 1000 cells/mL. SCC: Somatic Cell Counts. SCC N°: number of SCC.

of over counting, with thresholds and validity parameters close to those of the dairy cow [14, 16]. In both species, this kind of screening methodology can be adapted to operational purposes (detection for culling, drying-off treatment, etc.) and sometimes to IMI prevalence assessment [90]. Usually, in field conditions, a few iSCC values are available per lactation. In these cases, priority can be given to sensitivity, specificity, or predictive values in order to compensate for the decrease of overall efficiency.

6. TREATMENT

In small ruminant field conditions, clinical and subclinical IMI treatments must be distinguished, the average treatment cost per animal being high in comparison with the culling value and the expected recovery. In (per)acute clinical cases, the first purpose is to save the animal's life and, possibly, to save the diseased halves. In some areas, antibiotherapy is more and more used at drying-off, particularly for milk cellular quality control [22, 114].

Most of the published papers report clinical observations and/or recommendations adapted from results obtained in the cow; control-case studies are rare. Moreover, due to the general lack of specifically designed treatments for small ruminants, those for cattle are used (very few treatments are labelled for ewe and goat).

6.1. Clinical mastitis

No results from a controlled trial are available on the efficacy (i.e. bacteriological and clinical cure) of parenteral or intramammary antibiotherapy. A clinical study about tilmicosin treatment of ovine staphylococcal IMI and mammary dermatitis recently reported a complete symptomatology decrease five days after a single dose (10 mg/kg); this treatment seems to also have induced a cessation of milk shedding from days 5 to 7. *S. aureus* and CNS showed greater in vitro susceptibility to tilmicosin than to various other antibiotics [104]. Pharmacokinetics of various antibiotics have been studied after parenteral administration in the ewe and goat. Therapeutical schemes have been proposed but the efficacy has not been published so far: tobramycin (25 mg/kg) or apramycin (20 mg/kg) twice a day by intravenous route; enrofloxacin (5 mg/kg) or norfloxacin (10 mg/kg) once a day by the intramuscular route; tiamulin (25 mg/kg) twice a day by the intramuscular route; florfenicol (20 to 25 mg/kg) twice a day by the intravenous or intramuscular route [155]. Under field conditions, beta-lactamines and macrolides are still widely used via the intramuscular route, as recommended in former studies [154].

As in cattle, complementary treatments may be implemented in the severe cases [14, 47]. The economic interest of these treatments and also of new antimicrobial drugs (fluoroquinolones) use remains to be evaluated.

6.2. Subclinical mastitis

The efficacy of drying-off intramammary antibiotherapy depends on consider-

*ing bacteriological cure or prevention. The overall cure rate ranges from 65 to 95.8% in the ewe [1, 35, 64, 86, 87] and from 50 to 92.5% in the goat, whose dry period is shorter [7, 54, 89, 112, 114]. The efficacy is better for CNS than for *S. aureus* infections. These cure rates are higher than spontaneous cure rates (see Sect. 3.1.2.).*

The preventive interest remains to be discussed, particularly in ewes, considering the length of the dry period. Most bacteria isolated from colostrum are not isolated later in lactation (Berthelot, unpublished results). The new subclinical IMI rate at lambing or kidding is therefore difficult to estimate (two successive bacteriological examinations are necessary). This rate is probably low, between 5 and 20%; very large data sets are therefore statistically needed to identify a possible positive preventive effect of the drying-off treatment [1, 7, 17, 35, 87, 89].

Different treatment strategies are performed regarding the target for antibiotherapy. Additionally to the dry period length in ewes, three other small ruminant peculiarities should be taken into account: the large herd size, the production cycle synchronisation inducing a collective drying-off, and from a pathological point of view, the low drying-off and peri partum IMI incidences. Consequently, a selective drying-off therapy (concerning the infected udders only) may be preferred to a systematic one. The limitation of the number of treatments allows an easier implementation for a lower cost and, overall, reduces the utilisation of antibiotics and the risk of 'iatrogenic' udder contamination [22, 54]. Udder examination and iSCC or CMT are helpful to select the ewes and goats requiring an antibiotic treatment. Drying-off antibiotherapy is generally performed once a year for the 'whole' flock, but early dried females (including subclinical IMI) mostly remain untreated [22]. For these females, antibiotherapy must not be implemented at this time by the intramammary route since the teat duct is sealed by a keratin plug; the parenteral route

is the only usable one. An elegant possibility is to perform two (or three) successive sessions of intramammary infusion according to the respective lactation durations.

The intramuscular antibiotherapy (drying-off) would be an interesting route, particularly in large flocks, because it allows an easier implementation and a reduction of the 'iatrogenic' risk of udder contamination [155]. Nevertheless there is a lack of published controlled trials. According to field observations, two successive injections at drying-off are needed to possibly obtain a significantly higher cure rate than that of the untreated animals.

6.3. Risks associated with intramammary treatment

6.3.1. Clinical outbreaks

The development of drying-off intramammary antibiotherapy has been associated in sheep and goats with a few outbreaks of (per)acute to chronic clinical mastitis caused by opportunistic pathogens, especially *P. aeruginosa* (at the peri-partum period and sometimes at the beginning of dry period) or *A. fumigatus* (at the peri-partum period) [13, 66, 76, 110, 111, 117]. In most cases, the hygiene precautions had not been strictly respected at the time of implementation: teat-end disinfection, partial and atraumatic introduction of the cannula, infusion of a complete syringe in each udder-half, teat antisepsis. Certain specific risk factors possibly existing at the time of drying-off are now identified: liner contamination originating from *P. aeruginosa* multiplication in machine residual water, use of wet bedding or mouldy forage contaminated by *A. fumigatus*, etc. (Bergonier and Berthelot, unpublished results).

6.3.2. Residues

The risk of antibiotic residues in milk after intramammary treatments is poorly documented.

During lactation, the infusion (three syringes per udder-half at 12 h intervals) of a cattle-labelled formulation containing amoxicillin, clavulanic acid and prednisolone led to the detection of residues up to 136 h in the ewe and 112 h in the goat after the last infusion [30, 31]. In a similar study, cloxacillin residues were detected in goat's milk up to 156 h after the last infusion [61]. These results justify the 7-days withdrawal period prescribed by the European regulation after an out-of-label intramammary treatment in lactating small ruminants.

After drying-off treatments of dairy ewes with a cattle-labelled formulation containing penicillin (300 000 IU), nafcillin (respectively 100 and 109.2 mg) and dihydrostreptomycin (respectively 100 and 125 mg), residues were detected at lambing in four (respectively five) ewes out of 190 (respectively 25) and no more after three (respectively 5) days [35, 84]. In goats receiving the same formulation, residues were found at kidding in some females whose dry period had been shorter than two months; no more residues were detected after seven days [85]. These results confirm previous data [54], who found residues in only 1 out of 34 treated goats after a 107-day dry period.

Considering the dry period durations, it can be assumed that the risk of residues in milk is virtually null at lambing and, a fortiori, at the first milk delivery after a one to two-month nursing period. On the contrary, in the goat, the risk at kidding is present, particularly in cases of a shortened dry period (shorter than two months); in the latter conditions, a 14-day withdrawal period is justified, as recommended in the cow.

7. DISEASE CONTROL

7.1. Vaccination

No literature relative to recent advances in IMI vaccination is available since the

publication of a recent review [14]. Briefly, regarding *S. aureus*, vaccine preparations based on bacterial cell extracts, capsular polysaccharides and/or toxoids, products of virulence regulation genes, adhesins, etc. have been proposed to prevent murine or ruminant IMI. Attempts at immunisation with these preparations give generally insufficient results. In ewes, a liposome-immunopotentiated exopolysaccharide vaccine gave promising results [6]. Inactivated vaccines and autovaccines are commercially available and widely used for ewes and goats in several countries. Information about their efficacy in large scale controlled trials would be required.

7.2. Preventive general management

7.2.1. Control of bacteriological sources

The control of animal sources first targets intra-mammary carriage (primary sources). This is mainly performed by drying-off treatment of subclinical and mild chronic IMI and by culling the animals who experienced acute or severe chronic IMI: udder asymmetry, diffuse hardness, abscesses, etc. (abscesses must be differentiated from cysts, generally located in declivitous position sagittal and close to the cisterns) [131]. After drying-off antibiotherapy, those animals still presenting chronic signs at the beginning of the subsequent lactation should be culled.

Mammary cutaneous carriage might be controlled by pre-milking teat dipping; this measure is generally not used, due to herd sizes and milking routines, and probably to the low incidence of bulk milk flora problems. In cases of staphylococcal dermatitis or contagious ecthyma, udder antiseptics, isolation of affected animals and their lambs, and sometimes antibiotherapy should be performed. The suckling lamb's mouth and naso-pharynx carriage is important for staphylococci and *M. haemolytica* [3]. Its limitation relies on several other factors including the application of recommenda-

tions regarding the pen and the stocking density.

The control of environmental sources first consists in applying the pen recommendations for its conception, maintenance and stocking density (primary sources). The pen management plays a key role in controlling environmental bacteria IMI, and indirectly helps to reduce staphylococcal pressure through controlling density and air humidity. These factors are likely to enhance air and cutaneous staphylococcal concentrations. Stocking density may represent a critical factor in housing; too small space allocations may adversely affect performances and health [137, 139]. For environmental pathogens, the greatest care should be taken in the control of ambient hygiene, especially through efficient litter management and ventilation systems [2]. This is of important concern in intensive production systems, particularly in goats.

Secondly, the liners must be replaced every year for rubber ones, every two years for silicone ones. The milking machine (liners, clusters, pipelines, etc.) must be cleaned disinfected twice a day with drinking water and following a validated procedure (accessory sources).

7.2.2. Control of bacteriological transmission during milking

The milking technique represents a critical point for IMI control, but the specific risk factors associated to each time of the milking routine – and the milking equipment – have not been completely characterised. Over- and under-milking and every factor leading to impact (brutal and prolonged stripping, cluster removal without vacuum cutting off) must be avoided.

Some authors underlined that preventive practices, including milking techniques, were required to provide an effective decrease of IMI rates [44]. Minimising air inlets contributed to significant improvement in udder health status as far as minor pathogens presumed infections were concerned.

Minimising mammary massage, machine stripping and avoiding the removal of extra-milk reattaching the teat cups is recommended, particularly for primiparous females. The effect of milking techniques on bSCC were only significant at the end of the lactation (the middle or long-term effect).

Post-milking teat antiseptics seems to allow a 30 to 40% decrease of new IMI in the goat. The reduction is more important at the beginning of lactation, when spontaneous incidence is higher [12, 126]. The positive effect on bSCC is also significant at the beginning of lactation [44]. Goat SCC analysis evidenced a decrease of primiparous early IMI and IMI recurrences [40]. In another trial using iSCC but no bacteriology for the efficacy assessment, no difference was found between the dipped (nisin) and undipped groups [114]. In dairy ewes also, teat-dipping or spraying is an efficient tool for reducing IMI incidence, above all in high prevalence situations (Berthelot et al., unpublished results). This preventive measure is not frequently used, but could be implemented for a limited duration, during the period at risk (the suckling-milking period or the beginning of milking after weaning) or for controlling a mastitis or teat dermatitis outbreak. Its precise effectiveness according to the antiseptic molecules and to the different prevalence conditions remains to be evaluated in small ruminants, mainly in the ewe.

Implementing a milking order consists in milking primiparous and/or healthy females first. In France, this heavy technique seems to be more frequent in goats than in ewes [44]. Its development may be associated with other advantages in goats regarding CAEV control, alimentation or reproduction. Implementing a milking order has been shown to be associated with a decrease of (1) presumed major pathogen associated IMI in primiparous goats, and (2) presumed minor pathogen associated IMI in multiparous goats [44].

7.2.3. *Limitation of receptivity and sensitivity*

The non genetic control of udder receptivity is in particular based upon the reduction of teat lesions and pain caused by inadequate settings of the milking machine.

A survey on standards for small ruminants milking installations used in different countries showed differences in ewes and goat husbandry leading to differences in requirements for milking equipment [24]. Effective reserve, vacuum pump capacity and pulsation characteristics are especially in question. The lack of available knowledge prevents from taking into account specific characteristics of species and breeds in construction and installations of milking machines. Annual checking and regular maintenance of milking machines constitute basic measures insufficiently applied since only 40 to 60% of the total number of machines are checked annually. Mostly, the purpose of the investigations was to study the influence of machine parameters on milking efficiency [53, 141].

Quantitative recommendations for small ruminant milking installations were recently explored [25], with references to specific equipments such as non conventional clusters, references to specificity of milking practices (attachment rates for example) and to typical lactation curves recorded in various species and breeds. These recommendations will evolve during the next years and favour the development of reliable analysis of machine milking impact on teat duct health.

The control of udder sensitivity should be based on dietary recommendations: limitation of sudden transitions or lack of balance. On the contrary, milk retention must be reduced through adaptation of the equipment to animal yield, teat size and flock size [53]: vacuum pump capacity, vacuum reserve, claws volume and position with regards to the liners. Automatic cluster removal systems, when they exist, must be carefully set.

7.3. Genetic control

The genetic basis of susceptibility to IMI has been established (moderate heritabilities and QTL for SCC). Effective inclusion of mastitis resistance in the breeding goal of Lacaune breed, based upon SCC, has been recently implemented in France [124]. Accordingly, breeders can choose their reproducer rams on mastitis resistance in addition to production traits (milk yield, fat and protein contents) and PrP genotype (scrapie-resistant genotype). Improvement of resistance to mastitis by selection, however, is a long-term process and only partially efficient. Therefore, proper sanitary management remains the main clue to mastitis control.

8. FUTURE PROSPECTS

To control small ruminant mastitis, future research should concern the following fields, dealing with applied or basic research.

The improvement of diagnosis, particularly IMI cytological detection in goats. Future research in this field should attempt to determine the exact impact on total and differential counts of CAEV, herd management factors (dietary or medical stresses, etc.) and physiological factors (lactation stage, estrus, etc.). Non infectious variation factors of SCC are unique in the case of goats in comparison with ewes and cows; the inflammatory and immune processes of the caprine udder are not completely understood. This point must be underlined since SCC are the only large scale available screening tests to be used for control programmes, and since maximum levels are held as standards by the dairy industry or health officials. Progress in this field could be achieved by characterising bacteriologically negative halves with high and low SCC, at different lactation stages, from cytological, biochemical and histopathological points of view.

The improvement of diagnosis would also need, especially in small ruminants, the development of rapid animal-side tests. The available tools are of limited interest: the foremilk visual examination and the California Mastitis Test can not be regularly and systematically applied to large herds, and the on-line electrical conductivity measurement needs efficiency improvement and technical adaptations to small ruminant milking machines.

The assessment of bacteriological cure and new infection rates during the dry period, with or without antibiotic treatment at drying-off, require additional information in order to define and accurately choose the differential treatment strategies. Controlled trials are needed to better determine the bacteriological cure after drying-off intramammary or parenteral (two successive injections) antibiotherapy, according to the herd effects (main pathogens, infections length). They will also provide information about the new infection rates at lambing/kidding, in order to rationalise the choice between systematic and selective treatments and to assess the potential interest of teat sealers. Internal teat sealers are presumably less interesting in small ruminants than in dairy cattle: the intramammary implementation is not easier than conventional treatments particularly for large flocks/herds, and the incidence of new IMI during the dry period is lower.

The assessment of preventive measures would be of particular interest regarding the key-procedure in small ruminant IMI control: the milking machine management and the milking technique. Progress concerning the former was published last year through quantitative recommendations for small ruminant milking machine installations [25]. In the field, these recommendations and the basic measures are insufficiently known and applied, and very different quantitative values are observed (particularly for pulsation rates). Moreover, additional work is needed to clearly identify the possible adverse effects of the equipment

and milking routines on the receptivity of the mammary gland and on the spreading of organisms.

The development of a new generation vaccine protecting against S. aureus IMI is an important need, this organism being the main causative agent of acute and peracute IMI. Immunity of the ovine and caprine mammary glands is still poorly understood. Possible means to achieve these goals have been reviewed [116].

The detection of the ovine genome regions involved in mastitis resistance began with the first results of a Quantitative Trait Loci (QTL) detection program based on purebred families of French dairy sheep breeds [133]. It showed that QTLs for SCS were detected on chromosome 6 and 16, allowing to locate the gene(s) that control resistance to mastitis in this species. Additional and complementary results may arise from another QTL programme, based on crossbreeding between Sarda and Lacaune breeds, that was implemented in 1999 in a Sardinian experimental flock [34]. Conversely to the previous project, the whole genome will be investigated (genome scan), and traits related to mastitis resistance will also include information on clinical mastitis occurrence in addition to SCC.

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