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Review

New advances in epizootiology and control of ewe mastitis

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Abstract

The lactation (or annual) incidence of clinical mastitis in the ewe is generally lower than 5%, while the prevalence of subclinical mastitis is variable and ranges from less than 10 to 50% or more. *Staphylococcus aureus* is the most frequent bacterium responsible for clinical mastitis (from 20 to at least 60%). Coagulase-negative staphylococci are the principal causative agents of subclinical mastitis (30–95%), mainly in dairy ewes. Somatic cell counts (SCC) represent a valuable tool for prevalence assessment and screening. At an individual level, the use of several successive SCC allows the efficient detection of subclinical mastitis and is a good predictor of persistance. Healthy udders regularly show a SCC value lower than 500 000 cells/ml throughout the lactation period; values for subclinically or chronically infected udders usually exceed one million cells/ml. At the flock level, bulk milk SCC can be used to determine the overall intramammary infection prevalence, with a good coefficient of determination ($r^2 = 0.845$). Using SCC or the California Mastitis Test, and clinical examinations, ewes to be culled or treated can be identified. Immediate or delayed culling and intramammary antibiotherapy at drying-off are the main measures for the elimination of intramammary infections. Drying-off intramammary antibiotherapy is increasingly being performed in dairy ewes, as it provides a good bacteriological cure rate. Prevention is mainly directed against infections involving mammary sources, and includes milking machine control, milking routine optimisation, and post-milking teat disinfection. Control measures should take into account the peculiarities of dairy sheep breeding (e.g. flock size, seasonality, cost–benefit ratios, etc.).

Keywords: Mastitis; Intramammary infections; Sheep; Staphylococci; Somatic cell counts; Control methods

1. Introduction

The term 'mastitis' means udder inflammation, whatever the origin, severity and evolution. Most often, mastitis is of bacterial origin. In the ewe, intramammary infections are mainly due to 'non-specific' bacteria, mycoplasmas or lentiviruses; the former are the sole subject of this review. From a clinical point of view, mastitis takes various forms: clinical mastitis is characterised by general signs (fever, anorexia, weakness, coma, etc.), or only local signs (udder inflammation and oedema, gangrena, asymmetry, sclerosis, abscesses, etc.) and functional signs (macroscopic or quantitative modifications of

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milk production). Subclinical mastitis is characterised by quantitative and qualitative functional modifications (especially an increase in somatic cell count).

Mastitis is important for three reasons:

- economic (ewe and lamb mortality, treatment costs, reduced milk production, reduced lamb growth, milk payment on cellular quality in certain areas);
- hygienic (risk of infection or intoxication of consumers by milk bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., etc.). The main use of dairy ewe's milk is traditionally cheese processing;
- legal (E.U. Directive 46/92, modified by Directive 71/94, defining milk bacteriological quality and mentioning cellular quality).

Consequently, the control of mastitis and of hygienic quality of milk is the main goal for dairy and lamb-producing, sheep-breeding organisations.

2. Descriptive epizootiology

2.1. Incidence, prevalence and persistance

2.1.1. Clinical mastitis

2.1.1.1. Incidence The lactation (or annual) rate of new clinical cases is often less than 5%, both in dairy and meat flocks. Epidemiologically, these cases are generally sporadic clinical mastitis.

In a few outbreaks (less than 1 or 2% of flocks), the incidence may be higher (exceeding 30–50%), causing mortality or culling of up to 90% of mastitic ewes in a flock (Watson and Buswell, 1984; Marco Melero, 1994; Kirk and Glenn, 1996; Bergonier et al., 1997; Lafi et al., 1998; Calavas et al., 1998). These epizootic or enzootic cases are mainly due to specific pathogens (see Aetiology)

Thus, clinical incidence is generally lower in sheep than in dairy cattle.

2.1.1.2. Persistence The persistence of clinical mastitis during lactation is rarely documented, and depends on the production type (milk versus meat), and on the technical and specialisation level. Traditionally, mastitic ewes are rarely culled; acute cases become chronic leading to a persistence of several months, or even for a whole lactation. Such persistence is frequent (1.5 to $>30\%$) and close to the incidence in extensive systems (Watson and Buswell, 1984; Kirk and Glenn, 1996). In dairy flocks, especially in the most intensive ones culling is more often performed after clinical cases, (Marco Melero, 1994; Bergonier et al., 1997).

Data on persistence during the dry period are also rare. In two lamb-producing flocks, Watson and Buswell (1984) reported a frequency of udder abnormalities ranging from 13% at weaning to 3% at lambing (corresponding to a persistence of 25%) in ewes treated at weaning with intramammary cloxacillin.

2.1.2. Subclinical mastitis

2.1.2.1. Prevalence In meat flocks, the prevalence of subclinical mastitis is estimated by means of bacteriological analysis of half-udder or udder milk samples, individual somatic cell counts (iSCC) or the California Mastitis Test (CMT). The reported prevalence ranges from 5 to 30% per lactation (Watson and Buswell, 1984; Marco Melero, 1994; Kirk and Glenn, 1996).

Estimated by the same methods in dairy ewes, the mean prevalence was about 20–30%, ranging from 7 to $>60\%$ per lactation (Marco Melero, 1994; Bergonier et al., 1997; Lafi et al., 1998).

Bulk milk somatic cell counts (bSCC) can also be used to assess the prevalence of subclinical mastitis at the flock level. In 73 year-flocks, corresponding to 21,280 French ewes (Lacaune, Manech and Basco-Béarnaise), Lagriffoul et al. (1999) studied the relationship between the annual arithmetic mean (weighted by milk quantity) of bSCC and the rate of subclinically affected ewes (i.e. showing at least two iSCC of >1 million cells per ml). The estimated prevalence was about 6, 13 and 26%, respectively, in

flocks in which the annual mean bSCC was about 0.3, 0.6 and 1.1 million cells per ml, respectively (see Collective diagnosis).

In some countries, bSCC are carried out every month in every flock (De Crémoux et al., 1997; Lagriffoul et al., 1996b). In 1998, the mean bSCC was about 1.8 million cells per ml in Sardinia (geometric mean; Ledda and De Santis, 2000), 1.5 million cells per ml in Castilla-León, 0.6 million cells per ml in the Spanish Basque Country (arithmetic mean; Gonzalo et al., 2000), and 0.65 and 0.7 million cells per ml in the Roquefort area and the Pyrénées–Atlantiques, respectively (geometric mean; Lagriffoul et al., 2000). In the dairy ewe, bSCC values and their correspondence to iSCC allow a good estimation of the prevalence at the area and flock level.

2.1.2.2. Persistence

Due to the poor detection and elimination of such infections during lactation, and to their frequent staphylococcal origin, subclinical mastitis can present a long persistence. In a monthly bacteriological survey of 338 udder-halves during the entire lactation period in two dairy flocks, 40% of subclinical infections persisted for at least 3 months (Bergonier et al., 1996a). These results were confirmed in four other dairy flocks ($n = 430$ udder-halves), in which 54% of infected halves exhibited a single infection lasting 4 months (S.D. 2.1 months). The total lactation period was 8 months. In 44% of infected halves, two or three successive and different infections occurred (Bergonier and Berthelot, unpublished data).

The persistence of subclinical mastitis during the dry period has been studied in Latxa (Esnal et al., 1994) and Manech, Basco-Béarnaise and Lacaune breeds (Bergonier et al., 1996b); an overall self-cure rate of 50–67% of udder-halves was estimated. These results are similar to those reported in meat ewes: 33–48% (Watson and Buswell, 1984; Hueston et al., 1989; Ahmad et al., 1992a). The interpretation of such results must take into account the duration of the dry period (3–6 months) and the peculiarities of the aetiology of intramammary infections in the ewe (see Aetiology).

2.2. Variation factors for rates of occurrence

2.2.1. Individual factors

2.2.1.1. Lactation stage The incidence of clinical cases varies with the lactation stage. In dairy ewes, higher rates are observed at the beginning of lactation (during suckling-milking when performed) and particularly at the beginning of exclusive milking, mainly for machine milking and infections due to *S. aureus*. The peri-partum increase in the incidence of clinical mastitis observed in dairy cattle is infrequent in the ewe.

Drying-off is most often obtained by spacing the milkings and slightly reducing feeding; an increasing incidence is rarely observed at this time.

Finally, in very rare and specific cases (mastitis due to mycotic agents or to *Pseudomonas aeruginosa*), a maximal incidence is observed at drying-off and/or at lambing, in relation to poor hygiene practices or environmental contamination (Bergonier et al., 1997; Las Heras et al., 2000; Leitner et al., 2001).

2.2.1.2. Lactation number (parity) An increased prevalence related to the lactation number has been reported (Kirk et al., 1980; Watson et al., 1990; Ahmad et al., 1992a; Fthenakis, 1994; Sevi et al., 2000; Leitner et al., 2001); nevertheless, such descriptive data must be interpreted cautiously.

2.2.1.3. Udder conformation and genetic factors The relations between udder morphology (especially teat placement and orientation), milkability and SCC have been studied in certain breeds (see Receptivity) (Arranz et al., 1989; Carta et al., 1999; De la Fuente et al., 1999; Marie et al., 1999). Effects of the improvement in milking aptitude on udder susceptibility to mastitis are the subject of investigation (Barillet et al., 2001).

2.2.2. Environmental factors

The incidence of 'non-specific' infections (staphylococci) is particularly related to the milking machine design and setting and to the milking routine (hand and machine milking).

Marco Melero (1994), using CMT to detect subclinical mastitis, observed a higher prevalence in hand-milked flocks (32.3%) than in machine-milked flocks (28.8%) in relation to the annual mean bSCC (847 and 664,000 cells per ml, respectively). Therefore, the incidence of acute mastitis and mammary abnormalities was higher in machine-milked flocks. These data have not been confirmed by other studies and must be interpreted cautiously, since differences between breeding systems and technical level must be taken into account.

Persistence is conditioned by the quality of mastitis detection and the application of control measures (treatment, culling) by the farmer.

3. Aetiology

3.1. Clinical mastitis

Table 1 presents the results of studies of clinical mastitis in dairy ewes. Despite some differences in the methods, these data show the high prevalence of *Staphylococcus aureus*: from 17 to 57% of isolated bacteria (mean value approximately 36%, excluding Mycoplasma isolations). Coagulase-negative staphylococci (CNS), considered as minor pathogens in the cow, were isolated in 10.3–52.6% of clinical mastitis. The frequency of Streptococci, Pasteurella (including *Mannheimia haemolytica*, formerly *P. haemolytica*), Enterobacteria and Corynebacteria is low.

Outbreaks due to *Streptococcus uberis* and *Streptococcus suis*, or to opportunistic pathogens such as *Aspergillus fumigatus*, *Pseudomonas aeruginosa*, and, more rarely, *Burkholderia cepacia* or *Serratia marcescens* have been reported in various countries (Tzora and Fthenakis, 1998; Rapoport et al., 1999; Las Heras et al., 2000; Berriatua et al., 2001) and have sometimes been observed in France (Bergonier, unpublished data).

S. aureus is also the main causative agent of clinical mastitis in meat ewes (20–94% of isolated bacteria). Pasteurellaceae are more frequent than in dairy flocks (6–43%); CNS (2–26%), Streptococci (3–26%), *E. coli* (2–12%) and Corynebacteria (1–37%) have also been isolated (Hauke, 1960; König et al., 1983; Watson and Buswell, 1984; Al Samarrae et al., 1985; Jones, 1985; Nizamglioglu and Erganis, 1991; Kirk and Glenn, 1996).

Finally, the main differences between ewes and dairy cows in the aetiology of clinical mastitis are the following:

- a preponderance of staphylococci, especially of *S. aureus*, responsible for peracute to subacute mastitis;
- a high prevalence of CNS, agents of acute to chronic (and subclinical) mastitis;
- a low frequency of Streptococci, Enterobacteria and Gram-negative bacteria in general.

3.2. Subclinical mastitis

Table 2 presents the results of studies of subclinical mastitis in dairy ewes. CNS are the most

Table 1
Prevalence and aetiology of clinical mastitis in dairy ewes

Authors/year	Country	Ewes (*n*)	Flocks (*n*)	Type of milking	Samples (*n*)	% clinical mastitis	Negative (%)	*S. aureus* (%)	CNS (%)	Strepto. (%)	*E. coli* (%)	Past. (%)	Mycopl. (%)	Others (%)
Kryzanowski et al., 1983	Poland	757	7	H	1267			**57.5**	15.6	14	2.9	4.3		5.5
Baysal and Kenar, 1989	Turkey	3627	13	H	57	1.7	7.55	**30.2**			18.8		43.5	7.5
Bor et al., 1989	Israel	88	1				45.5	**16.7**	50					33.3
Fthenakis and Jones, 1990	Greece	5498	36	H	148	11.4		**29.5**	18.6	1.5	1	1.5	37.3	
Gutierrez et al., 1990	Spain		61	H	126		15.1	**40.2**	10.3					49.5
Marco et al., 1991	Spain		15		187	18		**28.75**	52.5	13.7	2.5	2.5	0	
Marco Melero, 1994	Spain	45	22	M and H	48		27.1	**28.6**	20	17.1		11.4		22.9
Lafi et al., 1998	Jordan	1736	46		145	2.1	18.6	**28.8**	11.9	8.5	18.6	3.4		28.8

M, machine milking; H, hand milking; CNS, coagulase-negative staphylococci; Strepto., streptococci; Coryn., corynebacteria + *A. pyogenes*; Past., *Pasteurellaceae* spp.; Mycopl., *Mycoplasma* spp. The frequency of negative samples is expressed as the percentage of the total bacteriological analyses performed. The frequency of bacterial types is expressed as the percentage of total isolation.

Table 2
Prevalence and aetiology of subclinical mastitis in dairy ewes

Authors/year	Country	Ewes (*n*)	Flocks (*n*)	Type of milking	Samples (*n*)	Prevalence (% ewes)	Negative (%)	CNS (%)	*S. aureus* (%)	Strepto. (%)	*E. coli* (%)	Coryn. (%)	Others (%)
Fruganti et al., 1985	Italy	15		H	300		75	**51.8**	26.7	20			
Baysal and Kenar, 1989	Turkey	3627	13	H	288	7.05	36.1	**62.5**	13.6	6	6	3.8*	6
Bor et al., 1989	Israel	88		M	88	55	45	**93**	4	0.2	0.5		0.8
Deutz et al., 1990	Austria	404		M and H	404		81.2	**36.5**	49.5	9.7	2		2.2
Otto, 1991	Germany	315		M	622		58	**39.2**	1.6	17.8	2.6		38.8
Schoder et al., 1993	Austria	201	4	M		8.9	91.1	**59.6**	25.3	12.1			3
De la Cruz et al., 1994	Spain	466	12		932	36.7	74.8	**79.1**	4.1	1.5		0.5	14.8
Fthenakis, 1994	Greece	760	8	M		11		**40.9**	16.7	9	9		24
Mavrogenis et al., 1995	Cyprus	100			1066		91.2	**66**	22		8	1	3
Gonzalez-Rodriguez et al., 1995	Spain	734	18	M and H	1382		56.2	**62.5**	11.4	16.2	0.4		9.5
Stefanakis et al., 1995	Greece	99	6		198	34	78.3	**40**	37	10		3	10
Bergonier et al., 1996a	France	169	2	M	2271		80.1	**80**	2.8				17.3
Bergonier et al., 1996b	France	655	9	M	1310		85.6	**91.5**	5.3	0.5			2.6
Cosseddu et al., 1996	Italy	205	14	M and H	410		75.4	**68**	3	4			4
Eitam and Eitam, 1996	Israel	482	5	M	947		77.3	**66**	12.4	1	7.8	0.5	11
Lafi et al., 1998	Jordan		46		118		16.95	**25.3**	9.6	14.4	19.3	6	25.4
Las Heras et al., 1999	Spain	564	22	M and H	1128	34.6	79	**68**	3.8	13.5		10.1	4.6
Bergonier et al., 1999a,b	Italy, Spain, France			M and H	36910		80.3	**77.55**	5.3	4	1.5		11.5

M, machine milking; H, hand milking; CNS, coagulase-negative staphylococci; Strepto., streptococci; Coryn., corynebacteria + *A. pyogenes*; *, *A. pyogenes* only. The frequency of negative samples is expressed as the percentage of total bacteriological analyses performed. The frequency of bacterial types is expressed as the percentage of total isolation.

prevalent, ranging from 25 to 93% (mean value approximately 62%), followed by *S. aureus* (3–37%), mainly isolated from infections that became chronic (less severe). Streptococci, *E. coli* and Corynebacteria are less frequent.

Among CNS, *S. epidermidis* is the species of the *Micrococcaceae* family more frequently isolated in Sarda, Latxa, Manech, Basco-Béarnaise and Lacaune breeds (Bergonier et al., 1999a).

In meat ewes, CNS are less frequent (12–34%). *S. aureus* represents 1–58% of isolations, while Streptococci and Pasteurella are more prevalent: 2–42 and 0.2–17%, respectively. *E. coli* and Corynebacteria are as frequent as in dairy ewes (Hauke, 1960; Al Samarrae et al., 1985; Maisi et al., 1987; Watkins et al., 1991; Ahmad et al., 1992a; Burriel, 1997).

Finally, the main aetiological characteristics of the subclinical mastitis of ewes are:

- the predominance of Staphylococci, CNS being very frequent;
- the limited role of Streptococci, Enterobacteria and other agents.

In conclusion, staphylococci are the main causative agents of clinical and subclinical mastitis in dairy and meat ewes.

4. Analytical epizootiology

4.1. Sources and associated factors

4.1.1. Sources

The main sources (primary sources) of staphylococci are clinically (chronically) and subclinically infected udders and infected teat injuries or viral lesions (Orf, i.e. Contagious Ecthyma). However, staphylococci, including *S. aureus*, are also carried by normal teat skin (without lesion), with a variable prevalence between flocks (Scott and Murphy, 1997; Bergonier et al., unpublished data).

Enterobacteria, Enterococci and Pseudomonas are found in the environment, the first two mainly in litter and *Pseudomonas aeruginosa* in water. *Manheimia haemolytica* is carried in the respiratory tract (nasopharynx and tonsils), but can survive in the environment (Ziluga et al., 1998). *Aspergillus fumigatus* and other fungi are environmental agents

(mouldy forage, wet bedding, litter, air). Finally, *Streptococcus uberis* and *Arcanobacterium pyogenes* are found in both animal (infected) and environmental (mainly litter) reservoirs.

Accessory sources are sites contaminated transitorily, most often after an infection developed in or from a primary source. The potential role played by the milking equipment (over-used liners, incorrectly disinfected clusters) and milker's hands during hand milking (Ziluga et al., 1998; Burriel, 1998) should be pointed out, especially for staphylococci.

4.1.2. Associated factors

For infected udders, the main factors contributing to bacterial persistence are the lack of early detection of mastitis (by udder palpation, foremilk inspection and SCC or CMT) and failure to apply elimination measures (treatment, culling). In a survey involving 77 flocks of the Roquefort area and the Pyrénées–Atlantiques (France, 1997), we observed that foremilk inspection was carried out occasionally, only in ewes exhibiting unusual behaviour or clinical signs, and that more than 66% of farmers did not used drying-off antibiotherapy.

Infected teat lesions (cutaneous staphylococcal infection, Contagious Ecthyma) represent also dangerous sources, as post-milking teat antisepsis is carried out only occasionally by less than 15% of farmers in France (Berthelot, unpublished data; see Treatment and Prophylaxis).

For extra-mammary sources, the persistence of bacteria is mainly due to poor maintenance or infrequent replacement of milking equipment and to the design and use of the pen (area and volume per animal, ventilation, possibility of parturient isolation). The humidity of the pen may enhance the multiplication of staphylococci on the skin and of fungi in the litter. Some bacteria (e.g. *P. aeruginosa*) may persist in the milking equipment even when the cleaning and disinfecting procedure has been correctly applied (Plommet, 1974; Jones, 1985; Bergonier et al., 1997).

4.2. Susceptibility factors

4.2.1. Receptivity

4.2.1.1. Animal factors Many studies on the non-genetic and genetic resistance to mastitis are currently conducted mainly in the cow, but also in the ewe and goat (Moroni and Cuccuru, 2001; Barillet et al., 2001). In the Lacaune breed, the heritability of the annual SCC score (SCS) was estimated to be 0.15; the heritability estimate varied from 0.04 to 0.12 from the first to the fifth test day. The phenotypic correlation was negative (−0.2) in relation to milk losses due to functional damage. The genetic correlation between milk yield and lactation SCS was slightly positive (0.15), but showed a strong evolution during lactation (38 flocks, 5272 first lactations). These results are consistent with genetic parameters in cattle (Rupp and Boichard, 1999) and suggest that high-potential ewes may present a higher susceptibility to mastitis. The genetic antagonism between milk yield and resistance to mastitis is lower than that between milk yield and milk composition. These results allow the consideration of mastitis resistance based on SCC as a criterion for the selection of sires (Barillet et al., 2001) (these results concern receptivity and/or sensitivity).

4.2.1.2. Environmental factors During lactation, the main factors for receptivity are the milking equipment, the milking routine and/or suckling. Thus, liners can induce repeated traumas, microhaemorrhages, congestions, erosions and even eversions if the vacuum level or the pulsation characteristics are inadequate; over-milking can induce teat duct lesions, and stripping can worsen this phenomenon.

During the suckling period, 'milk robber' lambs (orphan lambs and lambs underfed by mastitic ewes) may bruise the teats of the ewes they try to suckle.

Generally, drying-off is not a period of risk for new intramammary infections in the ewe, but teat duct integrity can be affected by traumatic canula insertion, leading to a higher receptivity.

4.2.2. Sensitivity

Milk retention can reduce the mechanical elimination of bacteria and polymorphonuclear activity, and facilitate the development of pre-existing infections. Milk retention can be due to an incorrectly working milking machine, inadequate stimulation, a too short milking time, stressful or painful milking (teat lesions, Contagious Ecthyma) and even udder morphology (teat placement).

Lastly, mastitis sensitivity can be influenced, as in the cow, by nutrition, especially by deficiencies in vitamin A and beta carotene, on the one hand, and vitamin E and selenium, on the other (Ronchi et al., 1996).

4.3. Transmission

4.3.1. Spreading

In the general case (staphylococcal infections), the main factor for spreading germs is milking. Germs are transported passively by liners, a phenomenon worsened by inadequate disinfection and replacement and/or lack of milking order. The milker's hands can also act as a vector for bacteria, especially during hand milking ('wet milking' consisting of hand and teat lubrication with foremilk or saliva).

During the suckling period, transmission occurs mainly by nursing, from the buccal carriage. A higher risk of spreading is constituted by 'milk-robber' lambs, as they can suckle an infected and a healthy udder successively.

4.3.2. Penetration

If we except systemic infection with an udder tropism (lentivirosis, mycoplasmosis, brucellosis, etc.), bacteria penetrate the udder by an ascending route (via the teat duct), even by active multiplication (after hand or machine milking) or by impact (machine milking). At the end of milking, udder massage and stripping induce air intake, leading to impact. Moreover, cluster removal by the milker may also induce impact (more often without cutting the vacuum off, as there is very little automatic removal). Thus, more than 50% of farmers perform an occasional or systematic stripping and 65% do not cut the vacuum off before cluster removal (Berthelot, unpublished data). However, one must keep in mind that the prevalence of mastitis is not significantly different between dairy (hand or machine milked) and meat flocks.

5. Diagnosis

5.1. Individual diagnosis

5.1.1. Clinical diagnosis

Acute and peracute mastitis are generally easily diagnosed by dairy ewe farmers who know their animals well, with a slight behavioural modification inducing a more complete examination.

Local signs should be detected by inspection and palpation of the udder (and lymph nodes). Functional signs should be observed, at the beginning of milking, by foremilk inspection in a black-bottom container; this is only occasionally carried out, as the number of ewes and the milking routine do not allow these dairy cow procedures to be performed readily (Berthelot, unpublished data).

The detection of chronic mastitis (udder asymmetry, sclerosis, abscess, etc.), by inspection and palpation, should lead to the identification of infected udders (which are sources of bacteria) in order to decide their treatment or culling. Such a diagnosis should be performed systematically at least at the beginning and end of lactation and before tupping, in dairy as well as in meat ewes (Watson and Buswell, 1984; Kirk and Glenn, 1996; Bergonier et al., 1997; Esnal et al., 1999).

5.1.2. Detection of inflammation

5.1.2.1. Somatic cell counts (i) Generalities: Milk SCC are a marker of udder inflammation, since leucocytes, the largest cell subpopulation in milk, originate from blood by chimiotactism and diapedesis as a reply to local agression. SCC present variations due to non-infectious factors, the effects of which are minor compared with those of intramammary infections. Such leucocytosis is due, in decreasing importance, to the lactation stage (beginning and end of lactation), the lactation number, the milk fraction, the number of lambs and various flock factors (Peris et al., 1991; Bergonier et al., 1996c; Lagriffoul et al., 1996a).

An instantaneous relationship between the isolation of bacterial species and SCC has been reported in various studies, concluding that the distinction between major and minor pathogens, formerly used in the cow, is not relevant to ovine mastitis. Thus, in the ewe, the geometric means of the SCC for udder halves range from 2.3 to 5 million cells per ml for *S. aureus* (mild chronic mastitis only), from 1.9 to 4.6 million cells per ml for *S. simulans*, from 1 to 1.5 million cells per ml for *S. epidermidis*, from 210 to 225,000 cells per ml for *S. xylosus* and from 130 to 150,000 cells per ml for sterile halves (Gonzalez-Rodriguez et al., 1995; Bergonier et al., 1997).

(ii) Practical use of SCC: threshold values for detection. Two types of approach for the practical use of SCC are proposed in the literature. The first proposes an instantaneous threshold value allowing the discrimination between 'healthy' and 'infected' udders or halves. The second proposes, as in the dairy cow, a 'decision rule' using several SCC during lactation and defining three classes ('healthy', 'doubtful' and 'infected'). The latter permits us to take into account the non-infectious variation factors and the dynamics of infection.

Studies proposing an instantaneous thresholds are based upon various methods for cell counting. With the fluoro-opto-electronic method, these thresholds range from 200,000 to 1 million cells per ml, with an efficiency (percentage of good decisions) ranging from 79 to 88% (Beltran de Heredia and Iturritza, 1988; Mavrogenis et al., 1995; Fthenakis, 1996; Romeo et al., 1996; McDougall et al., 2001). Romeo et al. (1998) proposed two thresholds: the first (140,000 cells per ml) to distinguish 'healthy' and the second (340,000 cells per ml) to distinguish 'infected' udders.

Studies proposing a 'decision rule' including a class of 'doubtful' udders have been conducted in France, in the Roquefort area and Pyrénées–Atlantiques. Methodologically, the 'decision rule' originates from a comparison of SCC performed every month during lactation (i.e. seven to eight values per animal) with the results of monthly bacteriological analyses of milk samples from udder-halves ($n=$ 2064). An udder is considered 'healthy' if every SCC (possibly except 2) is below 500,000 cells per ml, 'infected' when at least two SCC are over one million cells per ml and 'doubtful' in other cases. The efficiency of this rule is 74.5%, and the sensitivity and specificity are $>$80% (Bergonier et al., 1999b). These efficiency rates and thresholds are similar to those for dairy cow (Serieys, 1985). This kind of screening methodology can be adapted for operational purposes (detection for culling, drying-off treatment, etc.). Usually, under field conditions, a few iSCC values are available per lactation. In these cases, priority can be given to sensitivity, or specificity, or predictive values, in order to compensate for the decrease in overall efficiency.

In conclusion, in the ewe, as in the cow, SCC are a useful tool for the detection of subclinical intramammary infections, particularly when using several successive counts with two thresholds defining three classes.

5.1.2.2. California Mastitis Test The California Mastitis Test (CMT) allows a semi-quantitative evaluation of milk cell counts by observing the intensity of floculation after the addition of a reagent to the milk sample.

The concordance between CMT and SCC has been studied by comparing, for the same samples, SCC values to CMT scores (using the five-category grid of interpretation used in cattle). Thus, scores '0' (negative CMT) and '+/−' (slight non-persistent floculate) correspond to SCC values lower than 250,000 cells per ml (arithmetic mean); scores '++' (thick gel) and '+++' (thick and sticky gel) correspond to SCC values ranging from 500,000 to more than 900,000 cells per ml. The authors conclude that CMT is useful for classifying milk according to SCC, particularly when a simplified grid of interpretation is used: the efficiency ranges from 87 to 92% for scores '0' and '+/−', on the one hand, and '+', '++' and '+++', on the other (Ziv et al., 1968; Regi et al., 1991; Baumgartner et al., 1992; Marco Melero, 1994; Gonzalez-Rodriguez et al., 1996; McDougall et al., 2001).

The concordance between CMT and bacteriology is close to 80% (Marco Melero, 1994; Fthenakis, 1995; Gonzalez-Rodriguez et al., 1996), the sensitivity and specificity being 69.3 and 76.5%, respectively (Hueston et al., 1986). In some studies, the results suggest that the negative predictive value of CMT is greater than the positive predictive value (Maisi et al., 1987; Arranz and Beltran de Heredia, 1989; Marco Melero, 1994).

In conclusion, in the ewe, the CMT is a very useful, easy to perform and costless tool. It must be carried out before milking to take into account the SCC variations associated with the milk fractions (Peris et al., 1991). Its use is sometimes limited by farmers' difficulties in interpreting the results. It appears to be necessary to propose a simplified rule of interpretation (two to three classes), where farmers should be encouraged to repeat the test, to compare the results for the two halves of the same udder (to eliminate non-infectious bilateral varia-

tions) and to conclude only when the successive results agree.

5.1.3. Diagnosis of infection by bacteriological analyses of milk

Bacteriological examination of milk is a very useful tool in mastitis control programs. However, under field conditions, in dairy ewes and cows, such analyses are not performed systematically in the case of *sporadic clinical mastitis*, mainly for economic reasons. Moreover, a broad spectrum antibiotherapy is first performed.

In *clinical outbreaks*, milk from a representative sample of the affected animals must be analysed for bacteria, Mycoplasma and yeast, because the aetiological agents are diverse and the symptoms rarely pathognomonic. Under these conditions, a bacteriological diagnosis is necessary to determine more appropriate control measures.

5.2. Collective diagnosis (subclinical mastitis)

The relationship between bSCC and the prevalence of subclinical mastitis has been studied recently. Using the 'decision rule' proposed by Bergonier et al. (1999b), Lagriffoul et al. (1999) concluded that the annual arithmetic mean of bSCC (weighted by the volumes) is closely linked ($r^2 = 0.845$) to the proportion of ewes considered as 'infected'. Thus, the prevalence could range from 6 to 26% in flocks, in which mean bSCC are 300,000 and 1.1 million cells per ml. Considering an infected ewe with an iSCC of $>$340,000 cells per ml, Romeo et al. (1998) estimated the prevalence as 16 and 35% in flocks, in which bSCC of 250,000 and 1 million cells per ml, respectively. In these studies, the authors used different (annual mean vs. instantaneous values) and complementary methods.

6. Treatment

The literature on the treatment of mastitis in the ewe contains more general recommendations (extrapolated from data available for cattle) and clinical reports (without a control group) than controlled field trials; the latter mainly concern drying-off therapy. Currently, in France, only one treatment is officially indicated and authorised by the French Administration for dry ewe intramammary treatment (Longo and Pravieux, 2001). Under these conditions, many veterinary practitioners recommend, and many farmers use, treatments designed for the cow and for which withdrawal periods have not been determined for the ewe.

Such intramammary treatments must be applied with regards to strict hygiene conditions: complete milking, teat disinfection with an antiseptic towelette or a 70% alcohol-soaked compress, infusion of a complete syringe for each udder-half (and not half a syringe), not completely introducing the cannula in order to avoid teat duct traumatism, and teat antisepsis by dipping or pulverisation. If these hygiene conditions are not respected, and if the environment is contaminated by multi-resistant opportunistic pathogens (*A. fumigatus*, *P. aeruginosa*, etc.), outbreaks of clinical mastitis with mortality might occur at lambing (and sometimes at the beginning of the dry period) (Bergonier et al., 1997, Las Heras et al., 2000).

6.1. Clinical mastitis

Theoretically, the aim of 'in lactation' treatment is the clinical and bacteriological cure of infected halves and functional recovery. In fact, for acute and peracute mastitis, the aim is to avoid death and allow culling (immediate or delayed); in the case of subacute mastitis, functional recovery sometimes occurs, but culling will be considered at the end of lactation. In any case, affected ewes must be removed from the dairy flock until culling or complete recovery.

6.1.1. Antibiotic treatments

6.1.1.1. Intramammary treatments Due to the lack of controlled trials, no data for clinical and bacteriological cure after intramammary treatment is available for the ewe. Some clinical reports evoke 'recovery' and 'cure', but the assessment criteria are sometimes unclear. Under field conditions, this kind of treatment is not used frequently for economic and practical reasons.

6.1.1.2. Parenteral treatments Various studies have been published on antibiotic pharmacokinetics in the ewe, allow to propose therapeutical designs (the efficiency of which remains to be assessed): tobramycin, 25 mg/kg, twice daily by the intramuscular route; enrofloxacin, 5 mg/kg, once daily by the intramuscular route; norfloxacin, 10 mg/kg, once daily by the intramuscular route (Ziv and Soback, 1989). High doses of penicillin (Roguinsky, 1968) or spiramicin (Ziv, 1974) have been recommended; nowadays, beta-lactamines and macrolides are widely used under field conditions.

6.1.2. Complementary treatments

Antibiotic treatment may be completed by frequent milk-out of the affected gland and/or oxytocin (3 to 5 IU by the subcutaneous route), the parenteral administration of anti-inflammatory drugs and even perfusion in the most severe cases (East and Birnie, 1983). Each time, the economic costs of such treatments will be considered, with only a high genetic value justifying costly and/or time-consuming therapeutic measures.

6.2. Subclinical mastitis

The aims of drying-off treatments in the cow are bacteriological cure of infected udder-halves and prevention of new infections. This should allow control of milk quantity and quality in dairy flocks (Marco Melero, 1994) or improvement of numerical productivity in lamb-producing flocks (Hendy et al., 1981; Watson and Buswell, 1984; Kirk and Glenn, 1996). The spontaneous cure rate ranges from 33 to 67% (Watson and Buswell, 1984; Hueston et al., 1989; Esnal et al., 1994; Bergonier et al., 1996b).

6.2.1. Intramammary treatments

The published results show an overall bacteriological cure ranging from 65 to 95.8% (Watson and Buswell, 1984; Hueston et al., 1989; Ahmad et al., 1992b; Marco Melero, 1994; Longo et al., 1996; Longo and Pravieux, 2001).

The preventive efficiency is less easy to estimate, as the 'new' infection rate at lambing in untreated ewes is misappreciated and probably low (Hendy et al., 1981; Hueston et al., 1989; Ahmad et al., 1992b; Marco Melero, 1994; Longo et al., 1996; Kirk and Glenn, 1996).

From an operational point of view and whatever the route of administration, selective treatment (i.e. treatment of the infected ewes only) presents many advantages: reducing the number of animals treated, less and rational use of antibiotics, lower risk of hygiene default during intramammary infusion, easier implementation, and lower cost (Marco et al., 1999; Bergonier et al., 2001). The selection of animals to be treated will be based upon clinical examination (udder palpation) and the use of iSCC and/or CMT. As a matter of fact, the size of dairy flocks is increasing in several countries and drying-off antibiotherapy is performed once, at a flock level, at the end of the campaign. Ewes exhibiting an early decrease in milk production of infectious origin (subclinical mastitis) may be missed by these selective treatment procedures, when they need such antibiotherapy. A survey performed in 379 recorded flocks of the Roquefort area in 1999 showed that approximately 65% of the flocks used intramammary treatment at drying-off. The average percentage of ewes treated per flock was 64% (Bergonier et al., 2001).

6.2.2. Parenteral treatment

Parenteral treatment (mainly intramuscular) at drying-off could be an interesting alternative to local infusion for ergonomic (feasibility in large flocks) and sanitary reasons (lower risk of hygiene defaults) (Ziv and Soback, 1989). Unfortunately, results of controlled trials are missing, even though it seems that at least two successive injections would be necessary to attain the bacterial cure rates obtained after intramammary treatment.

6.3. Withdrawal period and residues

6.3.1. Intramammary treatment during lactation

In the ewe, Buswell and Barber (1989) studied the kinetics of antibiotic elimination in milk after three infusions, at 12 h intervals, of a formulation containing 200 mg amoxycillin, 50 mg clavulanic acid and 10 mg prednisolone. Residues were found up to 136 h after the last infusion, i.e. about 6 days (Buswell and Barber, 1989). These results suggest that, after intramammary antibiotherapy with a treat-

ment designed for the lactating cow, the milk withdrawal period must be longer for the ewe than for the cow. Under these conditions, the (at least) 7-day milk withdrawal period prescribed by European rules after out-of-label treatment appears fully justified.

6.3.2. Intramammary treatment at drying-off

In meat ewes, the infusion in each udder-half of a formulation containing 0.5 million IU penicillin and 250 mg streptomycin did not lead to residues in milk at lambing, after a 7-month dry period (Hendy et al., 1981). In dairy ewes receiving a bovine formulation containing 300,000 IU penicillin, 100 mg nafcillin and 100 mg streptomycin at drying-off, residues were found at lambing in only four of 190 treated ewes; after 3 days, no more residues were found (Lohuis et al., 1995).

Considering the length of the dry period in ewes, it can be assumed that there is almost no risk for residues in milk at lambing and, a fortiori, at the first delivery for human consumption, generally after a 1- to 2-month period of nursing.

7. Prophylaxis

7.1. Vaccination

A staphylococcal vaccine (exopolysaccharide), conceived and experimentally assessed in Spain (Amorena et al., 1994), was tested in a field trial in the Latxa breed. Two injections were performed during the month preceding and the month following lambing. The prevalence of intramammary subclinical infections was not significantly different between vaccinated and control groups during the entire lactation, but the frequency of clinical mastitis was reduced (Marco Melero, 1994).

To our knowledge, the efficiency of autovaccines has not been definitively proven in controlled trials. Stock vaccines exist, but a demonstration of their efficiency for the prophylaxis of intramammary infections remains to be performed. Currently, vaccination does not appear to be a decisive tool for the prevention of intramammary infections in the ewe on a large scale.

7.2. Sanitary prophylaxis

7.2.1. Source control

The elimination of existing infections relies on culling females affected by acute or chronic mastitis (clinical mastitis during lactation, udder asymmetry, diffuse hardness, abscess, etc.) (Saratsis et al., 1998) and drying-off treatment for subclinical mastitis (positive CMT and/or high values of iSCC). Ewes affected by clinical mastitis must be removed from the flock until culling. ‘Recidivist’ ewes, i.e. those still presenting chronic signs at the next lambing, should be culled before the beginning of milking.

The control of cutaneous sources consists of the prevention of teat injuries (viral or traumatic) and secondary bacterial contamination (by teat antisepsis). Flocks experiencing epizootics of Contagious Ecthyma or staphylococcal dermatitis may face important difficulties in controlling milk contamination and secondary mastitis due to *S. aureus* (parenteral antibiotherapy).

The liners must be replaced regularly, every year for rubber liners, every 2 years for silicone liners. The milking machine (liners, clusters, pipelines, etc.) must be cleaned and disinfected twice a day with drinking water and following a validated procedure.

The control of environmental sources mainly consists of applying the recommendations for the design and maintenance of the pen.

7.2.2. Transmission control

Although difficult, implementing a milking order (healthy females, especially primiparous, milked first) should be considered in flocks with recurrent problems of clinical mastitis (i.e. too high incidence of culling) or in those with very high bSCC.

During milking, measures should be introduced to prevent transmission. Annual checking and regular maintenance of milking machines constitute basic measures, which are insufficiently applied since only 40–60% of the total number of machines are checked annually (Billon et al., 1999). Other measures that can be implemented concern the milking routine: over- and under-milking and every factor leading to impact (brutal and prolonged stripping, cluster removal without cutting the vacuum off) must be avoided. In the case of hand milking, the milker’s hands must be cleaned before milking and teat

lubrication with foremilk or saliva should be prohibited.

Post-milking teat antisepsis is an efficient tool for preventing new infections, mainly in flocks with a high prevalence (Berthelot, unpublished data). Often considered as being too constraining by shepherds (because of flock size), this measure could be implemented for a limited duration, during the period of most risk (beginning of milking, generally after weaning) or when an outbreak of clinical mastitis or teat lesions occurs.

7.2.3. Susceptibility control

The control of udder receptivity is especially based upon the reduction of teat duct lesions caused by inadequate settings of the milking machine. The optimal operating parameters have been studied and present wide differences according to country (Billon et al., 1999; Molina-Casanova et al., 1999). Nevertheless, the following values may be proposed or are recommended: 36 kPa vacuum level, 180 pulsations per min and 50% pulsator ratio (Gonzalo and Marco, 1999). Variable pulsation ratio values are used in the field.

The control of udder sensitivity is based upon genetic and mainly non-genetic parameters, including certain aspects of nutrition (see Sensitivity), and the control of milk retention. Vaccination against Contagious Ecthyma contributes to the prevention of painful teat lesions. The equipment (vacuum pump capacity, vacuum reserve, claw volume and position with regard to liners, etc.) must be adapted to animal yield and flock size, even if there is a need for standardisation among European countries (Billon et al., 1999). Automatic cluster removal systems, when available, must be carefully set.

8. Conclusions

The characteristics of the intramammary infections of ewes, together with their breeding peculiarities, justify the definition and validation of specific control plans. Usually, the epidemiological model is 'reservoir' mastitis, with transmission of Gram-positive bacteria (mainly Staphylococci) during milking. Compared with dairy cows, the control of mastitis must focus on measures targetting animal carriage and optimisation of the milking routine and hygiene. Much new data is now available, as several research groups have improved our knowledge of aetiology, descriptive epizootiology, genetic factors, SCC, teat antisepsis, drying-off antibiotherapy, etc. Several aspects can be considered as still being poorly understood or rarely applied under field conditions on the basis of validated scientific results: ewe-side diagnosis, 'in lactation' antibiotherapy, vaccination, machine milking parameters and procedures, importance of certain nutritional elements, etc. Further investigations should be performed in these directions, particularly to ensure the bacteriological and chemical (residues) quality of milk.

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