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Risk factors and impacts of clinical and subclinical mastitis in commercial meat-producing sheep flocks in Quebec, Canada

Julie Arsenault, Pascal Dubreuil,
Robert Higgins, Denise Bélanger *

Faculté de Médecine Vétérinaire, Université de Montréal, 3200 rue Sicotte, C.P.5000, Saint-Hyacinthe, Québec J2S 7C6, Canada

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Abstract

We conducted a prospective observational study on clinical and subclinical mastitis in 30 commercial meat-producing sheep flocks from 2 regions of the province of Quebec, Canada. A total of 2792 ewes selected in late gestation were followed from lambing to weaning of lambs. The incidence of clinical mastitis for the total lactation period (average of 58 days) ranged among flocks from 0 to 6.6%, with a median of 1.2%. The most frequently isolated bacteria from the cases of clinical mastitis, in pure or mixed culture, were *Mannheimia haemolytica* (26%), *Staphylococcus aureus* (23%), and coagulase-negative staphylococci (17%). Incidence of clinical mastitis was higher in ewes that gave birth to 3 or more lambs and from the Estrie region, and was associated with an increase in ewe mortality, an increase in lamb mortality at the litter level, and a decrease in lamb's weaning weight for lambs born in multiple litter size or from ewes ≥4 years old.

Among 354 selected ewes with clinically normal udder at the end of lactation, 28.8% had potentially pathogenic bacteria isolated from milk. The most prevalent bacteria were *S. aureus* (9.3%) and coagulase-negative staphylococci (9.3%). The risk of having a positive culture in at least one half was different between the two regions. Prevalence of ewes ($n = 261$) with California Mastitis Test (CMT) positive result in at least one half was 24.1 and 14.9% using a cut-off of ≥1+ and ≥2+, respectively. Prevalence of culture-positive udder halves was 11.7% for CMT-negative compared with 53.6% for CMT 3+ halves. CMT status was positively associated with the isolation of coagulase-negative staphylococci, *M. haemolytica*, *S. aureus*, and various *Streptococcus* species, but not with other isolated bacteria. Additionally, prevalence of CMT-positive halves was higher in ewes from the Estrie region, aged of ≥4 years versus 1 year, having clinical mastitis previously detected in the lactation and/or with low body condition score. Lamb weaning weight was associated with CMT status of ewes, while

* Corresponding author. Tel.: +1 450 773 8521x1-8472; fax: +1 450 778 8120.
E-mail address: denise.belanger@umontreal.ca (D. Bélanger).

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weaning weight was not associated with milk culture results. More research is needed to understand the dynamic of milk SCC and IMI in ewes from meat-producing flocks, its economical impact and best ways to control it.
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1. Introduction

Mastitis in sheep is an important cause of mortality, decrease in production and premature culling (Jones and Watkins, 2000; Menzies and Ramanoon, 2001; Bergonier and Berthelot, 2003). Clinical mastitis in ewes has mostly been attributed to intramammary infection (IMI) by *Staphylococcus aureus* and *Mannheimia haemolytica*, and to a lesser extent to environmental pathogens, whereas subclinical mastitis has been mostly linked to IMI with coagulase-negative staphylococci (CNS) (Jones and Watkins, 1998; Menzies and Ramanoon, 2001). Infection by the maedi-visna virus has also been reported as an important cause of chronic subclinical mastitis in ewes (Cutlip et al., 1985; Menzies and Ramanoon, 2001; Arsenault et al., 2003b). The diagnosis of ovine subclinical mastitis is generally based on the demonstration of increased concentration of inflammatory cells and/or isolation of pathogenic bacteria from apparently normal milk in the absence of clinical abnormalities in mammary glands (Stefanakis et al., 1995; Clements et al., 2003). However, the pathogenicity of some bacterial species isolated from ewe milk is still unknown, and there is no consensus on the appropriate cut-off level for somatic cell count (SCC) for the detection of subclinical mastitis (Maisi et al., 1987; Heras et al., 1999; Clements et al., 2003). This absence of consensus might be related to difference in prevalence of various pathogens, because the level of increase in SCC depends on isolated species (Gonzalez-Rodriguez et al., 1995; Suarez et al., 2002).

Both clinical and subclinical mastitis have been associated with a reduction of milk yield and quality in ewes (Torres-Hernandez and Hohenboken, 1979; Heras et al., 1999; Albenzio et al., 2002). Reduction in milk yield has been suspected to decrease pre-weaning growth of lambs, and to increase lamb mortality from starvation in meat-producing sheep flocks (Watson and Buswell, 1984). In ewes with experimentally induced subclinical mastitis with CNS, the weight of lambs was significantly lower at 52 days of age compared to control ewes; these lambs also consumed more food (Fthenakis and Jones, 1990a). However, an observational study did not find any association between growth of lambs during their first 6 to 8 weeks of age and the degree of subclinical mastitis, in a management system where lambs had access to supplemental feed (Keisler et al., 1992). Another study found that the average daily gain of lambs from birth to weaning (~60 days) was significantly reduced in ewes with IMI detected at lambing and/or at weaning, but only when the intake of creep feed by lambs was limited (Ahmed et al., 1992).

The development or implementation of mastitis control programs in meat-producing commercial sheep flocks is dependent on the balance between their cost and the economical losses attributed to both clinical and subclinical mastitis. Such evaluation needs to consider both the prevalence and incidence of the disease within flocks and its impacts on productivity. The knowledge of risk factors and etiological agents involved are also important in order to recommend specific and efficient control measures. Many studies on clinical and subclinical mastitis have been done on dairy cattle, dairy goats and some in dairy ewes; however, the literature pertaining to meat-producing ewes is still limited. Thus, the specific objectives of our

study were to estimate in commercial meat-producing sheep flocks: (1) for clinical mastitis: the risk factors and impacts on ewe mortality, lamb weaning weight and lamb mortality; (2) for subclinical mastitis: the risk factors and impact on lamb weaning weight; (3) for subclinical mastitis, the association between CMT and bacteriological status.

2. Materials and methods

2.1. Selection of flocks

We conducted this study as part of a broader research project held in the regions of Estrie and Bas-St-Laurent, in the province of Quebec, Canada. We fixed the sample size at 10 flocks in the region of Estrie and 20 in the region of Bas-St-Laurent, which was proportional to the size of the sheep population in these areas. We restricted our study to flocks with $\geq$60 pregnant ewes in December 1999 because our interest was in flocks considered as a significant source of income for producers. All producers of both regions were informed of the project, and enrolment was done on a voluntary basis until the required sample size was reached.

2.2. Selection of ewes and clinical examination

Whenever possible, we selected 2 cohorts of 60 ewes in each flock, the first one in December 1999 and the other in August 2000. The sample size of 60 ewes was calculated to achieve objectives of a maedi-visna study performed in the first cohort within those flocks (Arsenault et al., 2003a), and was then used for the second cohort to have comparable sample size between cohorts. We allowed the same ewes to be selected in both cohorts. Selection was restricted to ewes in the last 2 months of gestation according to the producer. Pregnancy was determined either by ultrasonography, absence of estrus detection after mating and/or by recent udder development. We used a systematic sampling method for ewe selection, proportionally stratified for breed (each purebred was a category, and all crossbreds were pooled in one category), age (1, 2–3 and $\geq$4 years categories) and pens. For each pen, ewes were grouped with removable fences. Two persons located outside the fences did the selection at the same time by opening the fences and letting the sheep go out one-at-a-time. Ewes were selected systematically with a step calculated for the whole pen according to the number of ewes to be selected, with the nearest ewe selected first.

At selection time, a physical examination of ewes was done by a veterinarian or a technician under his/her direct supervision. A total of four people (3 veterinarians and 1 technician) participated in physical examination. Body condition score was evaluated by palpation of the lumbar region using a grading system from 1 to 5 with half scores, where 1 represented a very emaciated and 5 a very fat ewe (Radostits et al., 2000). Age was established by incisor examination. Schemas describing in detail body condition score and age categories were used during all the selection process for consistency across time and examiners. The head, neck, shoulders, hips and mammary glands were palpated to detect the presence of external abscesses. Breed information was provided by the producers.

2.3. Serology

For the first cohort only, the maedi-visna serostatus was evaluated as a potential risk factor for clinical or subclinical mastitis. A blood sample was collected at time of selection using jugular venipuncture. Blood samples were centrifuged and serum collected within 24 h of sampling. Sera

were frozen at −20 or −70 °C for a maximum of 4 months prior to analysis. An ELISA using gag and transmembrane-gst recombinant proteins was used to detect maedi-visna antibodies as described (Power et al., 1995). Cut-off was established at the mean optical density plus 3 standard deviations from a panel of 200 known negative serum samples. Sensitivity of this test relative to whole-virus-antigen ELISA was 97.4%, while specificity was 99.4% (Power et al., 1995). This test was performed by the Canadian Food Inspection Agency Laboratory in St-Hyacinthe, Quebec.

2.4. Flock follow-up

The first cohort was followed from ewe selection in December 1999 up to 1st May 2000, and the second cohort from ewe selection in August 2000 up to 15 December 2000. During the follow-up, producers had to record information about lambing (date, litter size, lamb's gender and birth weight), rearing (adoption status, date of lamb mortality) and weaning (date, weaning weight). Recording forms were provided and explained to producers at time of ewe selection. Producers were also asked to note any ewe mortality, culling and drug treatments, and to bring all selected ewes that died during the follow-up period to the nearest diagnostic laboratory of the Ministry of Agriculture, Fisheries and Alimentation of Quebec (MAPAQ) within 12 h of death. Producers received a financial compensation for the transport of dead ewes to laboratory, where a necropsy using standard procedures was performed free of charges for producers.

During the ewe's lactation, flocks were visited every 3 weeks by one of two persons (a veterinarian and a technician) up to the end of the follow-up periods. At each visit, recording forms were checked and corrected with the producer, and scales used by producers were calibrated using a 4.5-kg weight for newborn-lamb scales, and a 22.7-kg weight for weaning-lamb scales.

2.5. Clinical mastitis

Producers were asked to record cases of clinical mastitis in selected ewes and to collect milk sample before treatment. We defined clinical mastitis as the presence of a warm, painful and swollen udder and/or milk with abnormal aspect. The milk sampling procedure was explained in detail in a form given to producers at time of ewe selection. In summary, the udder had to be clean and dry, producers had to disinfect the teat with alcohol pads from the distal part to the udder, to discard the first stream of milk, to re-disinfect the teat and to fill a sterile tube provided without touching the inside and with the tube kept at 45°. The tube had to be identified and frozen at −20 °C immediately after collection. Milk samples were cultured within 1 month of collection at the Laboratoire de Santé Animale of the MAPAQ in St-Hyacinthe, Quebec. A 10 μL aliquot of each milk sample was surface plated using a sterile loop on Columbia or TSA agar supplemented with 5% sheep blood and incubated at 35 °C for 24–48 h. Identification of bacteria was done as previously described (Roy et al., 2007). Plates on which 2 or more identical colonies grew were considered positive for the bacterium, with the exception of *S. aureus* and *M. haemolytica* for which one colony per inoculum was considered positive. Samples positive for ≥3 different bacteria were considered as contaminated samples at collection and excluded from statistical analyses.

2.6. Subclinical mastitis

Sampling for subclinical mastitis was done between 24th March and 4th April 2000. In each flock, whenever possible, 15 ewes were sampled among lactating ewes previously selected in the first cohort. This sample size was calculated as the maximal number of samples we could test

considering study funding, divided by the number of available flocks. Lactating ewes were selected in order to be approximately evenly distributed among pens. For each pen, ewes were examined one-at-a-time, and those belonging to the first cohort were selected until the sample size for the pen was reached.

A sample of milk was taken separately for each lactating gland by one veterinarian or one technician under its supervision using the procedure described for clinical mastitis cases. Ewes having signs of clinical mastitis were not sampled. Milk samples were kept on melting ice during transportation to the laboratory and then at 4 °C. Within 24 h of sampling, 1 mL of each milk sample was tested using the California Mastitis Test (CMT) as an indirect evaluation of the SCC. The degree of precipitation and gel formation was assessed subjectively by one of three persons, under the supervision of the same one for classification of ambiguous cases, and graded as: (0) no precipitate or trace precipitate; (1+) distinct precipitate/weak gel formation; (2+) distinct gel formation; (3+) strong gel formation (Hueston et al., 1986). The rest of the milk sample was frozen at −20 °C for 1–5 months prior to culture.

Bacteriology was performed at the Faculté de médecine vétérinaire, St-Hyacinthe, Université de Montréal. A 8 μL aliquot of each milk sample was surface plated using a sterile loop on TSA agar supplemented with 5% sheep blood and incubated at 37 °C for 24–48 h. Identification of bacteria was done as previously described (Flores et al., 1993; Roy et al., 2007). Plates on which 5 or more identical colonies grew were considered positive for the bacterium, with the exception of *S. aureus* and *M. haemolytica* for which one colony per inoculum was considered positive. We defined a culture as positive if the sample was positive for at least one bacterium. Samples positive for $\geq$3 different bacteria were considered as contaminated samples at collection and were excluded from statistical analyses.

2.7. Statistical analyses

In general, we used descriptive statistics to present data and we tested two-by-two associations with the Pearson chi-square test with exact computation (Freq procedure, SAS 9.1). Level of statistical significance for the interpretation of various tests was fixed at 0.05. Only statistical models respecting tested assumptions were presented.

2.7.1. Incidence of clinical mastitis

We evaluated the hazard of clinical mastitis within flocks and its 95% confidence interval (CI) for the first 70 days of lactation by 10-day intervals periods using the actuarial life table method (Lifetest procedure, SAS 9.1).

2.7.2. Risk factor analysis

Potential risk factors tested for clinical mastitis, culture status and CMT status are presented in Table 1. For clinical mastitis, we used a Cox proportional hazard model with robust sandwich estimate for the covariance matrix to account for the ewe clustering within flocks (Phreg procedure, SAS 9.1). Event was defined as the first occurrence of clinical mastitis during lactation. Right censoring was either the end of the cohort follow-up period or the end of ewe lactation because of lamb's weaning, ewe mortality or ewe culling. We first evaluated risk factors by univariable analysis. Factors with a *P*-value $\leq$0.25 (Type-3 Wald test) from univariable analysis were included in a full model. A backward elimination of covariates was done using a *P*-value $>$ 0.15 as criteria, and only if their removal did not change the value of coefficients of others variables in the model by more than 15%. We calculated hazard ratio (HR) and their 95%

Table 1
Descriptive statistics of variables tested in the clinical and subclinical mastitis risk factors models in commercial sheep flocks in Quebec, Canada, 2000

Variable	Categories	Risk factors model						
		Clinical mastitis ($n = 2675$ ewes)		CMT ($n = 261$ ewes)			Culture ($n = 342$ ewes)	
		n	% Cases	n	% $\geq$ 1+	% $\geq$ 2+	n	% Positive
Cohort	Winter	1615	2.0					
	Fall	1060	1.4					
Region	Bas-St-Laurent	787	3.1	212	21.7	11.8	225	22.2
	Estrie	1888	1.2	49	34.7	28.6	117	42.7
Age of ewe (year)	1	913	1.8	144	18.8	12.5	183	31.7
	2–3	890	1.5	65	24.6	13.9	74	24.3
	$\geq$4	872	2.1	52	38.5	23.1	85	28.2
Body condition score	<2.5	332	1.2	20	35.0	30.0	24	20.8
	$\geq$2.5	2343	1.8	241	23.2	13.7	318	29.9
Maedi-visna serostatus[a]	Positive	497	2.8	67	28.4	20.9	89	27.0
	Negative	1112	1.6	191	23.0	13.1	250	30.0
External abscesses	Yes	105	1.9	10	30.0	20.0	18	27.8
	No	2570	1.8	251	23.9	14.7	324	29.3
Litter size at lambing	1	1149	1.2	117	16.2	9.4	158	22.2
	2	1228	1.7	119	28.6	16.8	149	35.6
	$\geq$3	298	4.0	25	40.0	32.0	35	34.3
Days in milk[b]	<50			135	23.7	11.1	145	26.9
	$\geq$50			126	24.6	19.1	197	31.0
Clinical mastitis[c]	Yes			4	75.0	50.0	6	33.3
	No			257	23.4	14.4	336	29.2

[a] Ewes from the first cohort only (excluding four ewes with missing value).
[b] At time of milk sampling.
[c] Ewes with clinical mastitis previously detected during lactation.

CI (based on the standard normal distribution) to present results. The assumption of proportionality of the hazard was evaluated by plotting the log of the negative log of survival against time.

For the culture and CMT status, we restricted the risk factors analysis to ewes with both halves tested (either CMT or bacteriology). We built three models with different definitions: (1) CMT $\geq$ 1+ in at least one half; (2) CMT $\geq$ 2+ in at least one half or (3) positive culture in at least one half. We built two-level (flock, ewe) hierarchical random-intercept logistic models in MlWiN, using penalised-2nd order quasi-likelihood estimation procedure with no extra-binomial variation permitted. Factors with a P-value ≤ 0.25 (Wald test) from univariable analysis were included in a full model, and a backward elimination of variables was then done as for the clinical mastitis model. Odds ratio (OR) and their 95% CI (based on the standard normal distribution) were used for the final model interpretation. We evaluated the assumptions of normality, linearity and homogeneity of variance at the flock level by examining normal-probability plot of standardized residuals and plot of standardized residuals against the fixed part of predicted

values. The assumption of binomial distribution at lamb level was evaluated by estimating the extra-binomial parameter.

2.7.3. Impact of clinical mastitis on ewe and lamb mortality

We evaluated the impact of clinical mastitis on ewe mortality and pre-weaning lamb mortality at the litter level using retrospective matched cohort study design. For each model, an ewe with clinical mastitis was matched, whenever possible, with five control ewes without clinical mastitis. Selection of control ewes was done using a pseudorandom number generator (SAS 9.1). For ewe mortality, matching was done on cohort, flock, number of days in lactation and litter size at parturition. For lamb mortality, matching was done on cohort, flock, number of days in lactation, litter size at parturition and number of lambs alive at time (days in lactation) of clinical mastitis detection; litters in which one or more lambs were given in adoption or weaned in the 10-day period following the detection of clinical mastitis and litters born less than 10-day before the end of study follow-up were excluded. We used conditional logistic regression for matched data (Phreg procedure, SAS 9.1). The outcome was the ewe mortality (yes, no) or lamb mortality in the litter (yes, no), respectively, in the next 10 days in lactation following the detection of clinical mastitis by producers.

2.7.4. Impacts on lamb's weaning weight

For the study of the impact of mastitis (clinical or subclinical) on lamb's weaning weight, we excluded lambs that died before weaning or were adopted by other ewes, artificially reared, weaned after 80 days of age or born less than 80 days before the end of cohort follow-up period. This 80-day period cut-off was the 95% percentile of weaning age distribution. We restricted the model on subclinical mastitis to ewes with both halves tested in bacteriological and CMT.

We fitted three-level models (flock, ewe, lamb) with random intercepts and fixed effects using the Mixed Procedure of SAS 9.1. The dependant variable was the lamb's weaning weight. For the clinical mastitis model, the occurrence of clinical mastitis between lambing and weaning (yes, no) was included as the fixed effect of interest. For the subclinical mastitis models, bacteriological status and CMT status were studied. The bacteriological status was categorized as positive or negative, without consideration of the number of positive halves due to the low proportion of bilateral infection. For the CMT status, results were categorized as positive in both halves, positive in one half and negative in both halves. We built two models with $\geq$1+ or $\geq$2+ as cut-off for the definition of a positive CMT.

Continuous covariates included in all models were the lamb's birth weight and age at weaning, and categorical covariates were the age of ewe, body condition score of ewe, litter size at birth (1, $\geq$2), gender of the lamb and occurrence of lamb mortality or adoption in the litter before weaning (yes, no). From the full models, covariates with $P > 0.15$ from the likelihood ratio test were removed using hierarchical backward selection if their removal did not change the other coefficients values by more than 15%. For the clinical mastitis model, the first-order interactions of mastitis with litter size and age of ewes were tested one-at-a-time in the final main-effect model. For the subclinical mastitis models, the following interactions were similarly tested: litter size with CMT status, litter size with bacteriological status, CMT status with bacteriological status. The models' assumptions of normality, linearity and homogeneity of variance were evaluated using MlWiN 1.10 by examining the normal-probability plot of standardized residuals and plots of standardized residuals against the fixed part of predicted value for each level of variance.

3. Results

Thirty sheep producers agreed to participate to the study and their flocks constituted the first cohort. For the second cohort, only 25 flocks were included because 3 flocks had no lambing during the follow-up period, one flock was sold and one producer discontinued his participation for medical reasons. Size of the flocks ranged from 95 to 1707 ewes, with a median of 347. All flocks were located many kilometers apart from each other, and none were included in any mastitis program control or carried out antibiotic treatment at drying off. All ewes lambed inside the barn, and all lambs had free access to creep feeding during the pre-weaning period.

The median number of ewes we selected by flock (including both cohorts) was 116 ranging from 60 to 157. The target sample size (60) was not obtained for all flocks, either because not enough ewes were available for selection or because it was easier for the producer to follow more ewes. Twenty-three percent of selected ewes were Dorset, Polypay, Romanov, Canadian Arcott, Rideau Arcott, Hampshire, Border Leicester or Suffolk purebreds; others were either hybrids from these breeds or crossbred.

A total of 2792 ewes were selected and followed, including 299 ewes selected in both cohorts. Among these 3091 ewes-cohort, 2675 (87%) lambed. For 6% of the 2675 lambings, the exact length of lactation was unknown due to non-recording of weaning date by producer, mostly because weaning occurred after the end of the follow-up period. For all analyses pertaining to clinical mastitis, these ewes were given a follow-up period corresponding to the number of days between lambing and end of cohort study, up to a maximum of 50 days. A total of 57 (2.1%) ewes died during their lactation period out of the 2675 ewe-cohort. Thirty-four dead ewes were submitted in necropsy. The most common diagnoses were paratuberculosis ($n = 11$), respiratory tract infection ($n = 10$), listeriosis ($n = 3$) and acute mastitis ($n = 3$). Selected ewes gave birth to a total of 4583 lambs. On average, lambs were weaned at 63 days of age (S.D. = 13) at a weight of 21.3 kg (S.D. = 6.4).

3.1. Clinical mastitis

3.1.1. Description of cases

Forty-five cases of clinical mastitis during lactation were diagnosed by producers. Two more cases of mastitis unnoticed by producers were included in analysis, one diagnosed during subclinical mastitis sampling and one in necropsy. No ewe had more than one episode of clinical mastitis. Among ewes with clinical mastitis, 25 (53%) were treated with antibiotics only, 8 (17%) with antibiotics and anti-inflammatory drugs, 2 (4%) with anti-inflammatory drug only, 3 (6%) died and 1 (2%) was culled before any drug administration, and no treatment was recorded in flock registry for the remaining 8 (17%) cases. Milk from 36 cases of clinical mastitis was collected by farmers and submitted to the bacteriology laboratory. Results are presented in Table 2. One sample with CNS, *S. aureus* and *Streptococcus uberis* isolated was excluded from this table because of presumed contamination.

3.1.2. Incidence rate

The incidence of clinical mastitis by flock for the total lactation period (average of 58 days) ranged from 0 to 6.6 cases per 100 lactations, with a median of 1.2. The incidence of clinical mastitis by days-in-milk intervals is presented in Fig. 1.

Table 2
Distribution of cases of clinical mastitis according to bacteria isolated from milk, Quebec, Canada, 2000 ($n = 35$ ewes from 17 commercial sheep flocks)

Bacteria	Number of samples (%)
Pure culture	
Bacillus spp.[a]	1 (3)
Coagulase-negative staphylococci (CNS)	3 (9)
Enterococcus spp.	1 (3)
Mannheimia haemolytica	7 (20)
Staphylococcus aureus	7 (20)
Mixed culture	
CNS and *Staphylococcus aureus*	1 (3)
CNS and *Mannheimia haemolytica*	2 (6)
Culture-negative	13 (37)

[a] Other than *Bacillus cereus*.

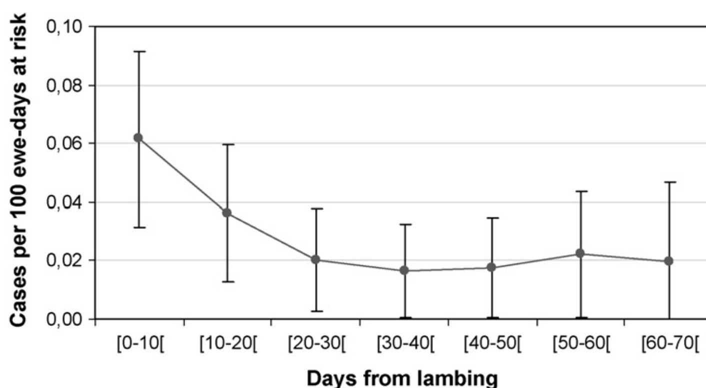

Fig. 1. Incidence of clinical mastitis with 95% confidence intervals in Quebec, Canada, 2000 (calculated at midpoint of intervals, $n = 2675$ lactations from 2424 ewes and 30 commercial sheep flocks).

3.1.3. Risk factors

The risk of clinical mastitis was significantly associated with region and litter size at birth (Table 3). No other variables were selected from univariable analysis (all $P \geq 0.28$). Ewes that gave birth to three lambs were significantly more at risk of clinical mastitis compared to ewe with

Table 3
Risk factors model[a] for clinical mastitis in Quebec, Canada, 2000 ($n = 2675$ lactations from 2424 ewes and 30 commercial sheep flocks)

Variable	Level	*b*	S.E.	Hazard ratio		
				Estimate	95%CI	*P(Wald)*
Region	Estrie	0.91	0.38	2.5	1.2,5.2	0.02
	Bas-St-Laurent			1.0		
Litter size	≥3	1.12	0.50	3.1	1.2,8.1	0.04
	2	0.23	0.37	1.3	0.6,2.6	
	1			1.0		

[a] Model likelihood-ratio $\chi^2 = 17.6$, d.f. = 3, $P < 0.001$.

single (HR:3.1, $P = 0.02$) or twins (HR:2.4, $P = 0.02$), but no difference in the risk of clinical mastitis was seen between ewes with single and twins lambs ($P = 0.53$).

3.1.4. Impact on ewe mortality

Among the 47 ewes with clinical mastitis recorded, six cases (12.8%) of mortality were recorded. Four ewes died the same day or the day after the clinical mastitis was noted, and mastitis as cause of death was confirmed for ewes ($n = 3$) submitted to necropsy. Two other deaths were noted 22 and 47 days, respectively, after the mastitis was noted. The cause of death was leg paralysis according to producer for the first one, and the other was a case of multiple internal abscesses diagnosed at necropsy, with different bacteria isolated from abscesses than from the mastitis milk sample. The occurrence of clinical mastitis was significantly associated with an increase in ewe mortality during lactation (OR = 4.4, 95% CI: 1.1, 17.8). The crude percentage of mortality in ewes selected for matched cohort analysis was 8.5% in ewes with clinical mastitis compared to 1.8% in ewes without clinical mastitis.

3.1.5. Impact on lamb's mortality

We matched 26 cases of clinical mastitis to control ewes for analysis; others cases had either less than 5 control ewes available for matching or did not have the 10-day period of follow-up requested. The median age of lambs at the beginning of the 10-day period of observation was 18 days. The crude proportion of litters experiencing lamb mortality in the 10-day period following clinical mastitis was 15.4% compared to 4.6% for control litters. In all but one litter, a maximum of one lamb died during the 10-day period of observation. The detection of clinical mastitis significantly increased the risk of lamb mortality (HR: 4.9, 95% CI: 1.03, 22.9). As an alternative model, we included all cases with 3–5 controls ($n = 35$); no major changes in estimates were observed (HR: 5.1, 95% CI: 1.2, 23.4).

3.1.6. Impact on lamb's weaning weight

A total of 2606 lambs were available for analysis. From these lambs, 207 (7.9%) had missing data for the birth weight, weaning weight or gender and were therefore excluded from the analysis. In the final main-effect model predicting lamb's weight, clinical mastitis was associated ($P = 0.05$) with weaning weight. Trends for interactions were then detected between clinical mastitis and litter size at birth ($P = 0.16$) and between clinical mastitis and age of ewes ($P = 0.11$); it was decided to keep those two models as two alternative final models (Table 4A). For each alternative final model, the effect of clinical mastitis was evaluated by computing the difference of least-squares means between ewes with clinical mastitis and those without for each level of the interaction (Table 4B). The reduction of lamb's weaning weight associated with clinical mastitis was statistically significant only for lambs from multiple litters and from ewes $\geq$4 years old. In ewes, a strong positive association was noted between the age and litter size; the percentage of ewes with multiple litter was 50, 59 and 67% for ewes aged of 1, 2–3 and 4 years old, respectively ($\chi^2 = 39.3$, d.f. = 2, $P < 0.0001$).

3.2. Subclinical mastitis

We obtained samples for subclinical mastitis from 27 flocks. Three flocks were excluded from the study because all lambs were already weaned at time of sampling. The number of selected ewes by flocks ranged from 3 to 16, for a total of 354 ewes. On average, ewes were sampled at 52 days in milk (S.D. = 15.6).

Table 4
Lamb's weaning weight models for clinical mastitis, Québec, Canada, 2000 ($n = 2399$ lambs from 1578 ewes and 30 flocks)

Variable	Categories	Model A[a]			Model B[b]		
		b	S.E.	*P*	*b*	S.E.	*P*
(A) Model estimates							
Intercept		−3.33	1.29	0.02	−2.87	1.56	0.08
Birth weight of lamb (kg)		2.10	0.09	<0.001	2.10	0.09	<0.001
Age at weaning (d)		0.22	0.01	<0.001	0.22	0.01	<0.001
Sex of lamb	Male	1.03	0.15	<0.001	1.04	0.15	<0.001
	Female						
Body condition score of ewe	<2.5	−0.85	0.29	<0.01	−0.84	0.29	<0.01
	≥2.5						
Mortality or adoption in the litter	No	−1.12	0.28	<0.001	−1.14	0.28	<0.001
	Yes						
Age of ewe (year)	≥4	0.27	0.24	0.26	−1.07	1.63	0.13
	2–3	0.57	0.22	0.01	2.56	1.67	0.51
	1						
Litter size at lambing	1	4.32	1.51	<0.01	2.20	0.22	<0.001
	≥2						
Clinical mastitis	No	1.91	0.80	0.02	1.50	1.21	0.21
	Yes						
Interaction between clinical mastitis and litter size at lambing	Mastitis = no, litter size = 1	−2.15	1.52	0.16			
Interaction between clinical mastitis and age of ewes	Mastitis = no, age = 2–3				−2.04	1.68	0.23
	Mastitis = no, age ≥ 4				1.36	1.65	0.41

Litter size at lambing	Clinical mastitis	LSM	Difference in LSM relative to absence of clinical mastitis		
			Estimate	S.E.	*P*
(B) Least square means estimates (LSM) for lamb's weaning weight (kg) by litter size at lambing and clinical mastitis status (Model A)					
1	Yes	22.9	−0.24	1.29	0.85
	No	22.7			
≥2	Yes	18.6	1.91	0.80	0.02
	No	20.5			

Age of ewes (year)	Clinical mastitis	LSM	Difference in LSM relative to absence of clinical mastitis		
			Estimate	S.E.	P
(C) Least square means estimates (LSM) for lamb's weaning weight (kg) by age of ewes and clinical mastitis status (Model B)					
≥4	Yes	18.8	2.86	1.13	0.01
	No	21.6			
2–3	Yes	22.4	−0.54	1.18	0.64
	No	21.9			
1	Yes	19.8	1.50	1.21	0.21
	No	21.3			

[a] Covariance parameter estimates: flocks = 5.5 (S.E. = 1.6), ewes = 6.2 (S.E. = 0.5), lambs = 8.2 (S.E = 0.4).
[b] Covariance parameter estimates: flocks = 5.6 (S.E. = 1.6), ewes = 6.2 (S.E. = 0.5), lambs = 8.2 (S.E = 0.4).

Table 5
Percentage of positive halves, ewes and flocks for the different types of bacteria isolated from clinically normal mammary glands in commercial sheep flocks from Quebec, Canada, 2000

	% Positive[a]		
	Halves ($n = 696$)	Ewes ($n = 354$)	Flocks ($n = 27$)
Arcanobacterium pyogenes	0.1	0.3	3.7
Bacillus spp.[b]	3.7	6.2	25.9
Coagulase-negative staphylococci	5.2	9.3	59.3
Corynebacterium spp.	1.3	2.5	14.8
Enterobacter cloacae	0.7	0.8	7.4
Klebsiella pneumoniae	0.6	0.8	3.7
Mannheimia haemolytica	0.4	0.8	7.4
Nocardia	0.4	0.8	11.1
Staphylococcus aureus	5.5	9.3	40.7
Streptococcus bovis	0.3	0.6	7.4
Streptococcus suis var. 9	0.4	0.6	7.4
Streptococcus uberis	0.3	0.6	7.4
Total	17.8	28.8	92.6

[a] A ewe was considered positive when at least one halve was positive, and a flock was considered positive when at least one ewe was positive.
[b] Other than *Bacillus cereus*.

A total of 697 milk samples from 354 ewes were cultured. Both halves were tested for 97% of ewes; for others, no milk could be extracted from one half, usually because the gland was not functional. CMT was performed on 550 milk samples from 289 ewes; for others, not enough milk was available to allow CMT testing in addition to culture. More than 60% of non-testing occurred in the first 4 flock sampled where we omitted to separate lambs from ewes at the time of sampling, so many lambs suckled their mother just prior to sampling.

The percentage of culture-positive halves, ewes and flocks by type of bacteria is presented in Table 5. From the 697 milk samples cultured, 82% were negative, 17% had only one bacterial type, 1% had two bacterial types, and 0.1% had three bacterial types (considered as contaminated and excluded from analyses). Among the 343 ewes with both halves tested in culture, 71% of ewes had a negative status, 23% of ewes were positive in one half and 6% of ewes were positive in both halves. Prevalence of CMT positive ewes is presented in Table 6.

3.2.1. Association between CMT and culture results

The percentage of culture-positive halves increased from 11.7% in halves with a negative CMT status up to 53.6% for half with 3+ CMT status (Table 7). The CMT status was positively

Table 6
Distribution (%) of CMT-positive ewes according to cut-off used, Quebec, Canada, 2000 ($n = 261$ ewes[a] from 25 commercial sheep flocks)

CMT-status	CMT cut-off	
	≥1+	≥2+
Positive in both halves	7.3	4.2
Positive in one half	16.9	10.7
Negative	75.9	85.1

[a] Only ewes with both halves tested are included.

Table 7
Percentage of culture-positive[a] halves (clinically normal udder) according to CMT status in commercial sheep flocks from Quebec, Canada, 2000

CMT	*n* halves	% Culture-positive
0	453	11.7
1+	38	18.4
2+	31	29.0
3+	28	53.6

[a] Considering only pathogenic bacteria.

Table 8
Distribution (number) of halves according to CMT status by culture-positive samples[a] for various bacteria isolated from clinically normal halves in ewes from commercial sheep flocks in Quebec, Canada, 2000 ($n = 550$ halves)

Bacteria isolated	CMT status (*n*)				% CMT ≥ 1
	0	1–2+	3+	Total	
Arcanobacterium pyogenes	0	0	1	1	100
Bacillus spp.[b]	18	0	1	19	5
Coagulase-negative staphylococci	18	10	1	29	38[c]
Corynebacterium spp.	0	0	1	1	100
Enterobacter cloacae	1	0	2	3	67
Mannheimia haemolytica	0	1	1	2	100[c]
Nocardia	3	0	0	3	0
Staphylococcus aureus	15	6	4	25	40[c]
Streptococcus bovis	0	0	2	2	100[c]
Streptococcus suis var. 9	0	0	3	3	100[c]
Streptococcus uberis	0	1	1	2	100[c]
No bacteria isolated	400	53	13	466	14

[a] All samples with *Klebsiella pneumoniae* cultured ($n = 4$) had missing value for CMT and were excluded.
[b] Other than *Bacillus cereus*.
[c] The CMT status (0 vs. $\geq$1) of the milk sample was significantly ($P \leq 0.05$, χ^2 test) associated with isolation of the bacteria. The percentage of CMT-positive samples in culture-negative samples ranged from 16 to 18% depending on the bacteria.

associated with the isolation of CNS, *M. haemolytica*, *S. aureus*, and various *Streptococcus* species ($P \leq 0.03$), but not with other isolated bacteria (Table 8).

3.2.2. Risk factors

Prevalence of culture-positive ewes was different between the two regions, and tended to be associated with litter size (Table 9). No other variables were selected from univariate analysis (all $P \geq 0.50$).

Depending on the cut-off used, significant associations of the CMT status with the region, age of ewes, body condition score, and/or detection of clinical mastitis previously in the lactation were detected (Table 10). In both models, the litter size was included, and the risk of positive CMT tended to be higher with an increasing litter size ($P \geq 0.07$). For the model using a cut-off $\geq$1+, all variables selected from univariate analysis were kept in the final model. For the one based on a cut-off of $\geq$2+, the clinical mastitis, age of ewe, days in milk and maedi-visna serostatus were selected from univariate analysis, but excluded from the final model during the selection procedure. No evidence of confounding between age of ewe and litter size was detected in the latter model.

Table 9
Risk factors model[a] for positive culture in at least one half, Quebec, Canada, 2000 (n = 342 ewes from 25 commercial sheep flocks)

Variable	Level	*b*	S.E.	Odds ratio		
				Estimate	95% CI	*P*
Intercept	Continuous	−1.73	0.31			
Region	Estrie	1.02	0.44	2.8	1.2, 6.6	0.02
	Bas-St-Laurent			1.0		
Litter size	≥3	0.61	0.47	1.8	0.7, 4.6	0.06
	2	0.67	0.29	1.9	1.1, 3.4	
	1			1.0		

[a] Extra-binomial variation parameter = 0.93, standard error = 0.07.

Table 10
Risk factors models[a] for positive California Mastitis Test in at least one half, Quebec, Canada, 2000 (n = 261 ewes from 25 commercial sheep flocks)

Variable	Cut-off ≥1+					Cut-off ≥2+				
	b	S.E.	Odds ratio			*b*	S.E.	Odds ratio		
			Estimate	95% CI	*P*			Estimate	95% CI	*P*
Intercept	−2.33	0.37				−2.87	0.44			
Region										
Estrie	0.96	0.48	2.6	1.0.6.6	0.04	1.48	0.58	4.4	1.4, 13.7	0.01
Bas-St-Laurent			1.0					1.0		
Age of ewe										
≥4 years	1.25	0.42	3.5	1.5,7.9	<0.01					
2–3 years	0.68	0.41	2.0	0.9,4.4						
1 year			1.0							
Litter size										
≥3	1.06	0.53	2.9	1.0,8.1	0.08	1.38	0.60	4.0	1.2, 12.9	0.07
2	0.60	0.35	1.8	0.9,3.6		0.62	0.45	1.9	0.8, 4.5	
1			1.0					1.0		
Body condition score										
<2.5						1.59	0.62	4.9	1.4, 16.5	0.01
≥2.5								1.0		
Clinical mastitis[b]										
Yes	2.85	1.34	17.2	1.2,237.3	0.03					
No			1.0							

[a] Extra-binomial variation parameters = (1) Cut-off ≥ 1+: 0.96 (S.E. = 0.09); (2): Cut-off ≥ 2+: 0.92 (S.E. = 0.08).
[b] Ewes with clinical mastitis previously detected during lactation.

3.2.3. Impact on lamb's weaning weight

From the 261 ewes with both halves tested in bacteriology and CMT, 447 lambs were born and followed. We excluded 8% of the lambs from the analysis because they died before weaning, 4% because they had been weaned before 40 days or after 80 days of age, and 4% because of missing data, resulting in 370 lambs from 247 ewes and 30 flocks.

Table 11
Lamb's weaning weight models[a] for subclinical mastitis, Québec, Canada, 2000 (n = 370 lambs from 247 ewes and 25 flocks)

Variable	Categories	Positive CMT: ≥1+			Positive CMT: ≥2+		
		b	S.E.	*P*	*b*	S.E.	*P*
(A) Model estimates							
Intercept		−1.05	2.53	0.68	−0.73	2.55	0.78
Birth weight of lamb (kg)		2.03	0.22	<0.001	2.04	0.22	<0.001
Age at weaning (d)		0.22	0.04	<0.001	0.21	0.04	<0.001
Sex of lamb	Male	1.57	0.37	<0.001	1.60	0.37	<0.001
	Female						
Litter size at lambing	1	1.58	0.55	<0.01	2.04	0.51	<0.001
	≥2						
CMT status	Positive in 2 halves	0.31	1.04	0.76	0.79	1.09	0.47
	Positive in 1 half	−2.02	0.69	<0.01	−1.70	0.70	0.02
	Negative						
Interaction between CMT status and litter size at lambing	Positive in 2 halves; 1 lamb	1.49	2.06	0.47			
	Positive in 1 half; 1 lamb	2.44	1.28	0.06			

Litter size at lambing	CMT status	LSM	Difference in LSM relative to negative CMT status		
			Estimate	S.E.	*P*
(B) Least square means estimates (LSM) for lamb's weaning weight (kg) by litter size at lambing and CMT status (model with positive CMT ≥ 1+)					
1	Positive in 2 halves	25.1	1.80	1.80	0.32
	Positive in 1 half	23.7	0.42	1.07	0.69
	Negative	23.3			
≥2	Positive in 2 halves	22.0	0.31	1.04	0.76
	Positive in 1 half	19.7	−2.02	0.69	<0.01
	Negative	21.7			

[a] Covariance parameter estimates: (1) Positive CMT ≥ 1+: flocks = 6.6 (S.E. = 2.5), ewes = 4.5 (S.E. = 1.3), lambs = 8.6 (S.E = 1.1); (2) Positive CMT ≥ 2+: Covariance parameter estimates: flocks = 7.1 (S.E. = 2.7), ewes = 4.6 (S.E. = 1.2), lambs = 8.5 (S.E = 1.1).

In the final main-effect models for lamb's weaning weight, the CMT status of ewes was associated with weaning weight, either when using a cut-off of ≥1+ ($P = 0.04$) or ≥2+ ($P = 0.03$). Only in the former model, an interaction tended to be present between litter size and CMT status ($P = 0.14$), and this interaction was kept in the model (Table 11). In both models, the weaning weight of lambs was significantly reduced in the presence of a unilateral positive CMT compared to a negative CMT, but only for multiple litter size if using a cut-off ≥1+. The culture status was not significant ($P \geq 0.81$) in the main-effect model, even if the CMT status was excluded ($P \geq 0.64$), and was thus was not included in the final models.

4. Discussion

We conducted this study in the Bas-St-Laurent and Estrie regions. We selected those areas because they had the highest number of sheep in production in the province of

Quebec. According to the last census before the study, the total number of ewes present in the studied flocks represented 11% of the total ewe population of Quebec (Gouvernement du Québec, 1999). We used a convenient sample of flocks, which might bias study results. However, to our knowledge, these flocks were not different from others in Quebec, and they were first selected for another purpose than this study (i.e. to conduct a seroprevalence study on maedi-visna infection, which was unknown for all producers but one). The risk factor analysis raised the hypothesis that the risk of clinical and subclinical mastitis might be higher in the Estrie region, suggesting that regional differences might be present within the province.

4.1. Bacteriology results

Coagulase-negative staphylococci, *S. aureus* and *M. haemolytica* were the main bacteria isolated from the cases of clinical mastitis, as reported by others (Jones and Watkins, 1998). Coagulase-negative staphylococci and *S. aureus* were also frequently isolated from cases of subclinical mastitis, as previously reported (Kirk et al., 1980; Bor et al., 1989; Watkins et al., 1991; Ahmed et al., 1992; Keisler et al., 1992; Gonzalez-Rodriguez et al., 1995; Jones and Watkins, 1998; Lafi et al., 1998; Croft et al., 2000). Coagulase-negative staphylococci are an heterogeneous group of bacteria often encountered in the environment of ewes and easily transferred between hosts (Burriel, 1998). The importance of staphylococci suggests that environmental contamination is important, and the control of ambient hygiene, particularly litter management and stocking density, could help to reduce the incidence of the infection (Sevi et al., 1999; Albenzio et al., 2002). Although bacteriological results from clinical and subclinical mastitis cannot be directly compared due to differences in sampling method and laboratory procedures, the predominance of CNS and *S. aureus* in clinical and subclinical mastitis suggests that a link might exist between clinical and subclinical mastitis caused by the same bacteria, either in term of common risk factors or in one state predisposing to the other. In ewes, the biological relationship between subclinical and clinical mastitis is still unclear (Bor et al., 1989).

4.2. Clinical mastitis

The classification of ewes for clinical mastitis might have been biased by producer evaluation, and some cases might have not been reported because it was extra work for the producers. However, producers were visited frequently and asked at that time if they had observed new clinical mastitis cases, and all laboratory procedures were done free of charge, conditions which should increase data quality. Although not statistically significant, a trend was seen toward an increased incidence of clinical mastitis in the first 3 weeks in lactation followed by a constant incidence until weaning. Another study reported an higher incidence for the first 2 days in lactation, but these results were not formally analyzed (Bor et al., 1989).

Ewes having three lambs or more were significantly more at risk of clinical mastitis. This had been previously reported and could be due to an increased stress in mammary glands by suckling lambs (Larsgard and Vaabenoe, 1993; Jones and Watkins, 1998; Lafi et al., 1998). An increased risk of teat contamination by lambs might also explain this association, because pathogenic bacteria may be present in the mouth of lambs and mechanically transmitted to ewes, as reported for *M. haemolytica* (Scott and Jones, 1998). On the other hand, it is possible that producers were

more attentive to udder health for ewes having three lambs or more, which could have overestimate the risk of clinical mastitis for those ewes.

We found a significant impact of clinical mastitis on ewe and lamb mortality, despite the treatment of most of these ewes by producers. Interestingly, no case of ewe mortality was noted among the seven ewes with clinical mastitis diagnosed on the lambing day. This could be the result of earlier detection and treatment of sick ewes by producers when it occurs at time of lambing, and/or to misinterpretation by producers of udder congestion in early lactation as clinical mastitis. All cases of lamb mortality in ewes with clinical mastitis occurred the same day or the day after the clinical mastitis was observed. The clinical mastitis was probably a cause of the lamb mortality instead of a consequence, because the observation of a lamb death by producers is probably more rapid than the incubation period of clinical mastitis. Ewes experiencing lamb mortality may have been more observed than others for clinical mastitis, considering the possible investigation of producers to find the cause of lamb's death; this might have overestimated the impact of mastitis on lamb mortality.

We found a significant association between lamb's weaning weight and clinical mastitis in the ewe, but only for multiple litter size at birth or older ewes ($\geq$4 years old), two characteristics highly correlated in ewes. We attribute the reduction in weaning weight to a reduction of milk production by the ewe, which is likely to have a greater impact as the number of suckling lambs increased and/or when the milk production is already compromised by older age, subclinical mastitis or udder chronic disease (Fuertes et al., 1998). Because the mastitis status was known by producers, it is possible that these lambs were given more supplementary milk or food by producers in order to reduce the impact of mastitis on growth. This was not quantified during this study, but should be considered in economical losses attributed to the disease. The impact of clinical mastitis on lamb's weaning weight is likely to be dependent on the period of lactation the clinical mastitis occurs. In our study, many cases of clinical mastitis were detected at mid to late lactation, and thus our results are possibly an underestimation of the impact of clinical mastitis occurring in early lactation.

4.3. *Subclinical mastitis*

A threshold of 625 cfu/mL was used to define a positive sample in bacteriology, which is an average value in comparison to other studies in ewes that used cut-off between 200 and 1000 cfu/mL (Hueston et al., 1986; Bor et al., 1989; Watkins et al., 1991; Gonzalez-Rodriguez et al., 1995; Lafi et al., 1998; Ariznabarreta et al., 2002). An exception was *S. aureus,* with one colony isolated considered significant as suggested by Ariznabarreta et al. (2002). The significance of various bacterial isolates is difficult to assess, because studies are lacking on the bacterial concentration expected in milk samples from infected gland, on the significance of mixed infection and on the pathogenic role of some isolated bacteria (Clements et al., 2003). In fact, some bacteria may have been present in the teat canal, without causing significant inflammation, and others may have been present as contaminant. Moreover, for CNS, for which all species were considered together and classified as pathogenic, an experimental study reported very different degree of pathogenicity for the ewe mammary gland depending of the species inoculated (Fthenakis and Jones, 1990b). In our study, the presence of *S. uberis*, *M. haemolytica*, *Streptococcus suis var. 9*, *Streptococcus bovis*, *Corynebacterium* spp. or *Arcanobacterium pyogenes* in milk samples was always associated with an inflammation of the mammary gland, although these observations are limited by the low number of positive samples for these bacteria. On the other hand, the isolation of *S. aureus* or CNS was significantly associated with an increased risk of CMT-positivity but

these bacteria were often isolated from CMT-negative milk samples. These bacteria can probably be present both as contaminant, infecting only the teat canal, or causing mastitis; another likely possibility is that the sensitivity of CMT for high SCC is not 100%. The other bacteria isolated (*Nocardia*, *Bacillus* spp. and *Enterobacter cloacae*) seem to have a minor role, if any, in the etiology of subclinical mastitis during the lactating period.

For the interpretation of CMT results, two different cut-offs were used to define a positive status. This was based on the work of Clements et al. (2003) and Suarez et al. (2002), who both reported an optimal sensitivity and specificity for predicting SCC when a cut-off between trace gel formation and distinct precipitation formation was used, considering a threshold of 10^6 cells/mL for SCC. This SCC threshold had been suggested to discriminate subclinical mastitis and the absence of inflammation based on experimental and natural infection in ewes (Fthenakis and Jones, 1990a; Fthenakis et al., 1991; Fuente et al., 1993; Burriel, 1997; Suarez et al., 2002), and is the most commonly used or recommended level as a diagnosis criteria for subclinical mastitis in ewes (Green, 1984; Jones and Watkins, 1998, 2000; Lafi et al., 1998; Menzies and Ramanoon, 2001; Bergonier and Berthelot, 2003).

The risk of positive-culture milk sample tended to be higher in ewes with litter size ≥ 2 lambs. As for clinical mastitis, it is plausible that the stress in mammary glands and likelihood of bacterial contamination increase with the number of suckling lambs. The exploratory analyses of CMT-positive risk factors bring some hypothesis, but further studies are needed for support. The risk of a positive CMT (cut-off ≥ 1) increased with the age of ewes; other studies also reported an increase in direct SCC or CMT with age (Gross et al., 1978; Lafi et al., 1998). The previous detection of clinical mastitis in the current lactation was also associated with an increased risk of CMT-positivity later in the lactation, even though the udder had returned to a normal appearance, but not to an increase risk in culture-positive result. This suggests that antibiotic treatments and/or natural healing were generally effective enough to eliminate or reduce the bacterial charge to an undetectable level, but that there was chronic damage to the mammary gland. The interval between the clinical mastitis detection and sampling for subclinical mastitis was 37 days on average (ranging from 11 to 69 days). Thin ewes were significantly more at risk of positive CMT status (cut-off ≥ 2); perhaps these ewes were more at risk of developing mastitis due to their poor general state, produced less milk and therefore were more at risk of over suckling by lambs, or that their low body condition score was a consequence of a condition increasing the risk of positive CMT test. Maedi-visna serostatus of ewes, despite its relatively high prevalence ($\sim$25%), was not a risk factor for positive CMT or culture, in contrast to the previous report of increased SCC in dairy goats seropositive to caprine arthritis-encephalitis virus (Nord and Adnoy, 1997).

The weaning weight of lambs, adjusted for confounding variables, was significantly associated with the CMT-status of ewes, but was not associated with the bacteriological status of milk. This is in agreement with another study, reporting that reduction in milk yield was more linked to SCC than to the bacteriological status (Heras et al., 1999). The reduction in lamb growth is attributed to a reduction in milk production secondary to mammary gland inflammation, whatever the cause of the inflammatory process. Moreover, the detection of pathogenic bacteria in the milk as an indicator of IMI is subject to misclassification bias: false-positive results may have occurred in the presence of contaminants, and false-negative results may be present as a result of sample freezing prior to culture, intermittent bacterial excretion or low level of gland bacterial contamination (Fthenakis and Jones, 1990a; Burriel, 1997; Clements et al., 2003). In addition, bacteria with unknown pathogenicity were excluded from the positive case definition but they might play some pathogenic role. It was not possible to evaluate the potential interaction between CMT and bacteriological status on lamb's growth in our study: too many categories were

present and their grouping would have included a large part of subjectivity (four categories were present at the half level considering both CMT and bacteriological status, leading to 16 different categories at the ewe level considering both halves). The reduction in lamb's weaning weight was only significant for ewes with one CMT-positive half, but not those bilaterally affected, which was unexpected. This could be related to the low number of ewes in the latter group, or to a residual undetermined confounding effect. The effect of CMT positive status on growth of lambs is likely to depend on the time it occurs during the lactation and on its duration; however, the dynamic of CMT status during lactation of the studied ewes was not determined.

Little is known about the persistence of high SCC across lactation in meat-producing sheep flocks. Without this information, it is not possible to evaluate if the suspected reduction in lamb's weaning weight of ewes with CMT-positive status would also be present in the next lactations. If the persistence of CMT-positive status is demonstrated, which is likely because older ewes were more at risk of positive CMT, addition of CMT status at weaning as a culling criteria and/or evaluation of various treatments at drying-off might be suggested. On the other hand, it might be possible to consider prophylactic injection of antibiotics before lambing, which had been shown in a clinical trial to increase weight of lambs in ewes >1 year old, an effect attributed to reduction in IMI (Croft et al., 2000). Any recommendation should be based on economical studies comparing overall cost of treatment relative to the detrimental impact of the infection or disease on productivity, and should also consider other important issues such as development of antimicrobial resistance, or certified organic status.

5. Conclusion

In order to reduce the impact of clinical mastitis, it could be advantageous to keep ewes with three lambs or more and older ewes within the same pens and to increase their surveillance, particularly in the first weeks of lactation. This would aim at treating ewes developing clinical mastitis promptly. Moreover, supplementary milk or food should be given to lambs of these ewes, both in order to possibly prevent clinical mastitis by reducing stress on the mammary gland caused by suckling lambs, and to reduce the impact on lamb mortality and growth following mastitis occurrence. Our results suggest that a positive CMT result could be linked to detrimental weight gain of lambs. More research is needed to understand the dynamic of milk SCC and IMI in ewes from meat-producing flocks, its economical impact and best ways to control it.

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