Bacteriology and somatic cell counts in milk samples from ewes on a Scottish farm

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Abstract

Milk samples from 50 sheep on a single Scottish research farm were collected weekly for 10 wk postpartum. Samples were analyzed for somatic cell counts (SCC) each week and bacteriologic culture was done for 7 of the 10 wk. A total of 492 udder half samples were cultured, of which 467 had corresponding cell count data. Statistical analysis on complete SCC and culture data showed no association between SCC and bacterial isolation, even when more than 10 colonies of a single bacterial species were present. Only 3.6% of the samples were simultaneously positive for high count (> 10 colonies from 0.01 mL of milk) of any one bacterial species and high SCC ($> 1 \times 10^6$/mL). The bacteria recovered were: *Staphylococcus equorum* (19 times), *S. xylosus* (7 times), *S. simulans* (6 times), *Streptococcus uberis* (3 times) and other streptococci (4 times), *Mannheimia (Pasteurella) haemolytica* (2 times), *Staphylococcus aureus* (1 time), *S. capitis* (1 time), and *Enterococcus faecium* (1 time). There was an association between the test day and SCC, with higher SCC values in the first 2 wk. In addition, significantly higher SCC values were found in the oldest animals compared to the other age groups.

Résumé

Des échantillons de lait provenant de 50 brebis d'une ferme expérimentale écossaise ont été prélevés à chaque semaine pendant 10 semaines post-partum. Le comptage des cellules somatiques (SCC) a été fait sur des échantillons prélevés à toutes les semaines alors que la culture bactérienne a été effectuée sur des échantillons de 7 des 10 semaines. Sur un total de 492 échantillons mis en culture 467 avaient des données de SCC correspondantes. Les analyses statistiques des SCC et des résultats de culture ne montraient pas d'association entre l'isolement bactérien et le SCC, et ce même si plus de 10 colonies d'une même espèce étaient présentes. Seulement 3,6 % des échantillons étaient positifs simultanément pour un dénombrement élevé d'une même espèce bactérienne (> 10 colonies/0,01 mL de lait) et un SCC élevé ($> 1 \times 10^6$/mL). Les bactéries retrouvées étaient : Staphylococcus equorum *(19 fois),* S. xylosus *(7),* S. simulans *(6),* Streptococcus uberis *(3) et autres streptocoques (4),* Mannheimia (Pasteurella) haemolytica *(2),* S. aureus *(1),* S. capitis *(1) et* Enterococcus faecium *(1). Une association a été notée entre le jour du test et le SCC, avec des valeurs de SCC plus élevées durant les 2 premières semaines. De plus, des valeurs de SCC significativement plus élevées ont été trouvées chez les animaux plus âgés comparativement aux autres groupes d'âge.*

(Traduit par Docteur Serge Messier)

Introduction

Microbial analysis and somatic cell counts (SCC) have been used to diagnose subclinical mastitis (SCM) in ewes. This disease has received little attention, particularly in places where sheep are raised for meat rather than milk. Subclinical mastitis is thought to have a prevalence of between 10% and 30% in lowland flocks in Southern England (1,2). This condition can affect milk yield and milk composition, with consequent effects on growth rates of lambs (3,4). Comparative studies on ovine SCM are hampered because of the lack of universally accepted standards for the diagnosis of this problem. Colony counts of various bacterial isolates in combination with a high SCC or bacterial colony count, or SCC alone, are used for case definition (5). Fthenakis (6) used the term SCM for cases where 0.01 mL of a milk sample yielded > 10 colonies of a single organism on Columbia Blood Agar, and simultaneously the sample had a SCC of $> 1.0 \times 10^6$ cells/mL. Other workers (7,8) have used lower threshold values for the diagnosis of SCM. In bovine mastitis, major pathogens are considered of relevance when fewer colonies are found. The National Mastitis Council (NMC) guidelines consider a single colony of *Staphylococcus aureus* or *Streptococcus agalactiae* significant in culture. Environmental streptococci must have 2 to 9 colonies to be considered significant, whereas, coagulase-negative staphylococci (CNS) must have > 10 colonies in pure culture to be considered relevant (9).

The objectives of this study were to gain information on levels of SCM in a population of ewes by SCC and bacteriological analysis of milk obtained by repeated sampling during a period of 10 wk postlambing, and to examine the association between culture status and SCC.

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Materials and methods

Fifty Scottish Blackface cross Border Leicester (Greyface) ewes of 2 to 7 y of age from Moredun Research Institute's farm located in Midlothian, Scotland, were used in this study. The group was randomly selected from a 500 ewe breeding flock set up to provide animals for use in Institute studies, as well as for commercial lamb production. To allow easy gathering for weekly milk sampling, the sheep used in the study were maintained as a single group. All ewes were housed in individual pens for approximately 24 h after lambing followed by housing in open pens for 3 to 7 d before being turned out to graze with their lambs for the remainder of the experiment. Ewes with clinical mastitis were not included in this study. In compliance with the United Kingdom (UK) Animals (scientific procedures) Act of 1986, all required approvals were obtained prior to the experiments. From 3 d postlambing, 10 to 15 mL milk samples were obtained from each udder at weekly intervals for 10 wk. The sheep were restrained in a sitting position in a cradle that exposed the udder. The foremilk was removed and the teats were then wiped with disposable alcohol impregnated swabs (Sagrosept wipes; Schulke and Mayr, Sheffield, UK) before a mid-flow milk sample was manually expressed from each udder into separate sterile 25 mL universal tubes (Bibby Sterilin, Staffs, UK). The samples were examined immediately or held at 4°C for less than 24 h. Samples for all 10 wk were examined for SCC using a Coulter counter (Coulter Counter ZM; Beckman Coulter, Buckinghamshire, UK). The machine was calibrated for bovine SCCs, rechecked prior to each sample run, and the samples processed in accordance with the methods described by the International Dairy Federation (10). Briefly, the 10 mL milk samples were fixed in 3.5% formaldehyde and diluted in 1:400 in a buffered isotonic saline solution (Lechesol; Apertech, Bedfordshire, UK), and a 0.5 mL aliquot was subsequently used for SCC measurement. To correct for sample dilution, the number of SCC/mL was obtained by multiplying the SCC data by 800 ([(Raw SCC $\times$ 400)/500] $\times$ 1000).

Samples obtained in the 1st, 2nd, 5th, 6th, 8th, 9th, and 10th wk were also examined for presence of bacteria by culture. A 0.01 mL sample of milk was plated on Columbia blood agar and MacConkey agar number 2 (Oxoid, Basingstoke, UK), using a calibrated loop. The plates were incubated aerobically at 37°C for up to 72 h, examined daily for the number and types of colonies, and the information recorded. Bacteriological interpretation was based on the NMC recommendation for bovine milk cultures (9). Briefly, samples were considered to have a major pathogen if they had 1 or more colonies of *Staphylococcus aureus*, *Streptococcus agalactiae*, or *Mannheimia (Pasteurella) haemolytica;* if they had 2 or more colonies of environmental streptococci growing in pure culture; or more than 10 colonies in mixed culture with one other species. Samples were considered to have a minor pathogen if greater than 10 colonies of CNS, corynebacteria, or other bacteria, excluding *Bacillus* species, were present. Samples with 3 or more species on a plate were considered contaminated. The bacteria were identified based on colony morphology; Gram's stain; and tests as required, for catalase, coagulase, oxidase, and finally, using API bacterial identification strips ("ID32 STAPH," "rapid ID32 STREP," "api Coryne," "api 20 NE;" bioMerieux sa, Lyon, France).

Table I. Culture results of 467 milk samples with high somatic cell counts (SCC)[a]

Growth/Bacterium	Number	%	Samples with High SCC	%
No growth	178	38.1	70	39.3
Contaminated with *Bacillus*	117	25.1	55	47.0
Major pathogen	10	2.1	2	20.0
Minor pathogen	36	7.7	15	41.6
Mixed	94	20.1	54	57.4
CNS < 10 colonies	20	4.3	13	65.0
Other	12	2.5	7	58.3

CNS — Coagulase-negative staphylococci
[a] SCC/mL = $> 1 \times 10^6$ for the sample

A total of 233 samples comprising all of the 2nd and 3rd wk (total 187), and 46 samples from the 10th wk (selected on the basis of SCC $> 1 \times 10^6$ in 1 or more previous weeks) were, in addition, selectively cultured for *Listeria monocytogenes* as per the methods described by Fthenakis and others (11). In brief, milk samples were cultured in *Listeria* broth with selective supplement (Oxoid CM 862, SR 141; Oxoid) and, after incubation at 30°C, subcultured onto supplemented polymyxin B-acriflavine-lithium chloride-ceftazidime-aesculin-mannitol (PALCAM) agar (Oxoid), and examined for *Listeria* colonies.

All data were transferred to a spreadsheet program (Quattro Pro, version 8.0; Corel Corporation, Ottawa, Ontario) and exported to a statistical package (Stata, version 7.0; Stata Corporation, College Station, Texas, USA) for analysis. The data entered into the program included the identification number of the sheep, year code, origin of the sample (left or right udder), laboratory identification number, sample date, SCC, bacterial growth, number of colonies, and bacterial identification.

Descriptive statistics for bacterial growth and SCC parameters were created. For analysis, SCC data was converted to the natural log (LNSCC) of the raw SCC value. Linear regression models were constructed to examine associations between the LNSCC and bacteria classified into 2 groups for the purpose of major and minor pathogens, as previously described. Analysis was done at 3 cutoff points of SCC (500 000, 1 million, and 2 million). Additional regression models examined the association between test day, udder half, birth year (ear tag code), and LNSCC.

Results

A total of 492 milk samples were cultured during the study period, of which 467 had corresponding cell count data. The results of culture are summarized in Table I. Of these, 178 (38.1%) were negative for bacterial growth and 117 (25.1%) were contaminated. Ten samples were positive for major pathogens, which included 1 *Staphylococcus aureus,* 3 *Streptococcus uberis,* 4 *Streptococcus* spp., and 2 *Mannheimia (Pasteurella) haemolytica.* Thirty-six were positive for minor pathogens, which included 19 *Staphylococcus equorum,* 7 *S. xylosus,* 6 *S. simulans,* 1 *S. capitis,* 1 *Enterococcus faecium,* 1 *Corynebacterium* spp., and

Table II. Summary of somatic cell count (SCC) data $\times 10^3$

Cell counts[a]	All weeks culture	Weeks with negative	Culture pathogen	Major pathogen	Minor pathogen
Number of observations	925	467	178	10	36
Mean	1876	2351	2285	1762	2293
	(6.98)[b]	(7.12)	(7.03)	(6.77)	(7.17)
Median	933	944	905	673	902
	(6.84)	(6.85)	(6.81)	(6.51)	(6.80)
Standard deviation	4053	4907	5366	3371	3067
	(0.84)	(0.92)	(0.92)	(0.97)	(1.01)
Minimum	113	174	213	371	256
	(4.73)	(5.16)	(5.36)	(5.92)	(5.45)
Maximum	50000	50000	47286	11329	14702
	(10.82)	(10.82)	(10.77)	(9.34)	(9.60)

[a] SCC/mL

[b] Linear scores

Table III. Somatic cell counts (SCC) compared to repeated bacterial isolation

Sheep per sample	Pathogen	Somatic cell counts $\times 10^3$ for the 3 weeks			Mean
		1	2	3	weeks 1 to 10
1655Z-L	*Streptococcus uberis*[a]	9063	467	444	1421
		(9.11	6.15	6.10)[c]	(6.99)
		1	5	9	
1317B-L	*Staphylococcus equorum*[b]	8186	564	480	1648
		(9.01	6.34	6.17)	(6.92)

[a] > 100 colonies week 1, > 50 colonies week 2, > 200 colonies week 9

[b] 10 to 30 colonies all 3 weeks

[c] Linear scores in brackets

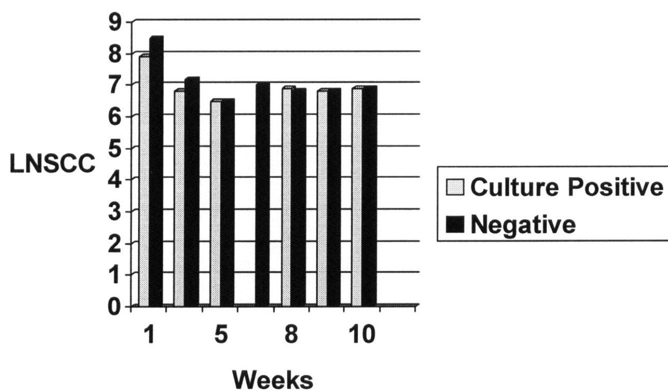

Figure 1. Natural log of raw somatic cell count (LNSCC) values versus culture results.

1 *Aerococcus* spp. Ninety-four samples had mixed growth with < 10 colonies of minor pathogen types, and 20 samples had < 10 colonies of CNS. Twelve others had growth of *Bacillus* (8 times), alpha-hemolytic streptococci (1), *Enterococcus* spp (1), and unidentified Gram-negatives (2). None of the 233 samples examined was positive for growth of *Listeria monocytogenes*.

The SCC value was $< 1 \times 10^6$/mL for most of the culture-positive samples, including 1 positive for *Staphylococcus aureus* and 1 positive for *M. haemolytica*. However, the mean SCC values for 10 wk, from the samples originating from the *S. aureus* and *M. haemolytica-positive* udder halves, were $> 1 \times 10^6$ (data not presented). Overall, only 17 (3.6%) of 467 samples were simultaneously positive for bacteriologic culture (major and minor pathogens) and SCC ($> 1 \times 10^6$ cell/mL).

Nineteen samples were positive (> 10 colonies) for *Staphylococcus equorum*. Of these, only 6 samples were positive by SCC, whereas 5 of the 7 *S. xylosus*-positive samples had high SCC values.

Regression analysis, using the 467 culture results with SCC data, did not reveal any association between the LNSCC and pathogens, either those considered major (n = 10) or minor (n = 36) (4 of the minor pathogens did not have SCC's). The SCC data are summarized in Table II. Figure 1 illustrates the LNSCC and the week for culture negative versus positive (major or minor pathogens). The mean SCC for all samples of the study, regardless of whether there was a corresponding bacterial culture was 1867×10^3. The mean SCC of samples that were bacterial culture-positive was 2351×10^3. The mean SCC of samples that were negative for bacterial growth was 2285×10^3. No association between bacterial culture positivity and SCC was found. The mean SCC value for major pathogens was 1762×10^3, and for the 36 samples positive for minor pathogens, it was 2293×10^3. There was no statistically significant difference (confidence level 0.95) between these values and the value for samples with no bacterial growth. There was an association between test day and LNSCC. Samples in the first 2 test weeks (mean =

3426.22×10^3) had higher SCC ($P < 0.01$) than those on the other test days (mean = 971.74×10^3). There was no association between SCC and udder half. No association was found between the SCC of noninfected glands and the infection status of the contra-lateral gland. When ear tag code (birth year) was examined, the oldest animals (birth year X) had higher SCC than those in other age groups ($P < 0.05$).

Milk samples from the left udder half of 2 ewes were repeatedly (3×) positive for 2 species of bacteria (Table III). *Streptococcus uberis* was isolated (> 50 colonies) for 3 wk from samples obtained from the left udder half of ewe 1655Z-L, but the SCC was positive only in the 1st wk. Similarly, a second ewe was positive for *Staphylococcus equorum,* with positive SCC only in the 1st wk. However, the mean SCC values for 10 wk in both cases were $> 1 \times 10^6$/mL.

Discussion

The diagnosis of SCM on the basis of a selected SCC value or a particular colony count of bacteria is problematic because workers have used different cut-off values. There is a lack of an accepted "normal" value, and there is no agreement on the accepted number of colonies as significant or vice versa. A high SCC value in the absence of bacterial growth on blood agar may be due to bacteria, such as mycoplasma, or due non-bacterial causes, including physiological factors. Perinatal SCC values were higher, which could be explained by these physiologic factors. It is also possible that SCC values may change with the stage of bacterial infection. In the present study, milk from the same sheep was sampled for 10 wk for SCC, and bacterial culture examined 7 times during the 10-week period. Statistical analysis on complete SCC and bacterial culture data showed no association between SCC and bacterial isolation, even when more than 10 colonies of a fully identified single bacterial species were present. However, 3.6% of 467 samples were simultaneously positive for a bacterial pathogen and SCC. Using the definition of SCM as the presence of both bacteriologically positive and SCC positive results, Jones and Watkins (2) reported a 11.7% rate of SCM during lactation among the lowland flocks in England and Wales. The 3.6% prevalence rate of SCM among the 50 ewes at the Firth Mains farm in this study is quite low compared to the study mentioned above. To determine the infection rate in a herd, both bacteriological status and SCC in milk should be taken into account, especially when prevalence within a herd is not known (12).

Thirty-three of 492 (6.7%) samples were positive for CNS and *S. equorum*, followed by *S. xylosus* and *S. simulans*. Coagulase-negative staphylococci are the most frequently isolated bacterial group from ewes with SCM and during lactation (4–7). Although *S. equorum* isolates in the present study had excellent identification under the API Staph Ident system, reports on this species are scanty and it has only been isolated from goat milk (13). *Staphylococcus epidermidis, S. simulans, S. sciuri,* and *S. xylosus* were the most common isolates from SCM in ewes in Greece (6). Burriel (14) reported that *S. simulans, S. xylosus,* and *S. hyicus* were the predominant species of CNS in the milk from meat ewes, and *S. epidermidis* was the predominant species in the milk of dairy ewes. However, in the present study, the API Staph system, which is recommended by the National Mastitis Council (15) for species-level identification of CNS, was used. It should be noted that some variations in speciation of CNS can occur when different commercial identification systems are used (16).

The majority of our isolates were CNS. In a study conducted by Gonzalez-Rodriguez (7) CNS were the most frequently isolated bacterial group, although these bacteria gave rise to significantly lower SCC values compared with coagulase-positive staphylococci and streptococci. Fthenakis and Jones (3) induced SCM in ewes with a strain of *Staphylococcus simulans,* and noted an increase in SCC following infection. In our study, 6 samples were positive (> 10 colonies) for *S. simulans*. No association was found between culture positivity for this bacterium and increased SCC, though in 1 sample the SCC was high. Similarly, some of the samples positive for *Staphylococcus equorum* and *S. xylosus* had high SCC values, but a statistical association was not found between culture positivity and SCC. It is possible that species of CNS and strains under each species have varying pathogenic properties. Some reasons for a lack of correlation between bacterial culture positivity and SCC have been pointed out and include the coexistence of bacteria in the absence of inflammation, lack of actual parenchymal infection, and use of different equipment for performing SCC (2,6,17).

Only 1 of the 2 samples that were positive for *M. (P.) haemolytica* had a high SCC. *Mannheimia (Pasteurella) haemolytica* strains from different sources, including isolates from cases of SCM, have been shown to vary in virulence, and in their ability to establish colonies in the mammary gland (18).

The SCC data in Table III seems to question any stable relationship between presence of bacteria in large numbers and SCC. Despite high counts of *Streptococcus uberis,* in samples obtained within 3 wk, there was no high SCC except for the 1st wk. Further, there is strong evidence of intermittent excretion of the bacterium because of the fact that the samples from only weeks 1, 2, and 9 were positive (50 to 200 colonies), while samples obtained in the other weeks were bacteriologically negative. This could also be interpreted to be self-cure and reinfection. Fhenakis (6) has discussed the dynamics of SCM, including intermittent excretion of bacterial pathogens in milk from ewes. Ewes have been shown to develop SCM with *Mycoplasma bovigenitalium,* characterized by intermittent *Mycoplasma* excretion and low milk cell levels (19). The absence of increased SCC with the occurrence of heavy bacterial growth with *Streptococcus uberis* is puzzling and needs further research.

Naturally occurring ovine SCM is a matter of concern and recent reports implicate bacteria other than the conventional pathogens, including *Listeria monocytogenes* (11) (which was not isolated from any samples in the present study), *Corynebacterium mastitidis* and *C. camporealensis* (2 newly reported species) (20,21), *Streptococcus parasanguinis* (22), and *Burkholderia cepacia* (23).

Although the occurrence of *Bacillus* spp. in milk samples from ewes can be high at times, the contamination rate of 25% found in the present study needs further investigation as to the possible role of the environment. Watson and Buswell (24) noted that *Bacillus* spp. is predominant in the milk samples from ewes on pasture.

In conclusion, this study did not demonstrate any valid association between positivity for bacteria by culture and SCC in the 492 milk samples from ewes studied during a period of 10 wk of lactation. If

one considers simultaneously positive results for significant bacterial growth (> 10 colonies of a particular species from 0.01 mL of milk) and high SCC (> 1×10^6/mL), only 3.6% of samples were positive for SCM, a very low prevalence among the ewes in this study. There was an association between the test day and SCC, with higher SCC in the first 2 wk. In addition, significantly higher SCC values were found in the oldest animals compared to the other age groups.

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