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Research paper

Intramammary infections and somatic cell counts in meat and pelt producing ewes with clinically healthy udders

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ABSTRACT

Mastitis in sheep is common and is important both from an economic and from a welfare point of view. It can be either clinical or subclinical, bacterial or, more seldom, lentiviral. There are no data on the national prevalence of subclinical mastitis (SCM) or intramammary infection (IMI), and causative pathogens, in ewes in Sweden.

Diagnosis of SCM and/or IMI is based on inflammatory indicators, measured in milk and additional bacteriology. Somatic cell count (SCC) is the most widely used indicator of SCM and/or IMI and can be measured either directly or indirectly with e.g. California Mastitis Test (CMT). However, the use of SCC as an indicator for mastitis and/or IMI in ewes is not fully evaluated, especially not in meat- and pelt producing herds. The aim of this study was therefore to investigate the prevalence of IMI and bacterial panorama in ewes with clinically healthy udders, in meat- and pelt producing herds in Sweden. Moreover, we wanted to define a cut-off for SCC and CMT for detecting ewes with IMI that would be suitable for use under field conditions.

Udder half milk samples (n = 2134) were collected at weaning and lambing from meat- and pelt producing ewes (n = 773), in 22 farms in Sweden. Only clinically healthy animals were included in the study. Milk samples were analysed for bacteriology and SCC and were given a CMT score.

Intramammary infection was found in 30% of the ewes and in 14% of the udder half milk samples. No bacteria were found in 74% of the milk samples, and mixed flora was found in 12% of the samples. Among the 287 milk samples where IMI was identified, coagulase negative staphylococci (CoNS) were most prevalent (58%) followed by *Staphylococcus (S.) aureus* (9%) and *Mannheimia (M.) haemolytica* (6%). Among the 165 CoNS findings *S. simulans* was the most common (26%), followed by *S. warneri, S. equorum, S. xylosus, S. haemolyticus* and *S. chromogenes*. A CMT score of ≥3 and a SCC of ≥500,000 cells/ml at weaning or CMT score ≥3 and a SCC of ≥400,000 cells/ml after lambing gave the highest possible Se at the same time as the highest possible Sp for identifying udder halves with IMI and may be used for screening for IMI in Swedish meet and pelt producing herds.

To conclude, one third of Swedish meat- and pelt producing ewes without clinical signs in the udder had IMI in one or both udder halves at weaning and or after lambing, and CoNS was the most common bacterial finding. A high SCC (≥400,000–500,000 cells/ml) was associated with IMI and a difference in having a high (CMT 3–5) or low (CMT 1–2) between udder halves can be used as an indicator of IMI under field conditions.

1. Introduction

Mastitis in sheep is economically important due to premature culling of animals (Watson and Buswell, 1984) and costs of treatment. Moreover, it adversely influences welfare of affected animals (Gelasakis et al., 2015) and causes decreased milk production, which can lead to decreased growth rates and increased mortality in lambs (Watson and Buswell, 1984; Fthenakis and Jones, 1990; Moroni et al., 2007; Huntley et al., 2012; Holmoy et al., 2014; Grant et al., 2016). In milk producing herds, mastitis is also of importance for food safety and processing properties (Klinger and Rosenthal, 1997; Leitner et al., 2004; Silanikove et al., 2014). In meat- and pelt producing herds, mastitis is probably not linked to any human health risks since milk is only consumed by the offspring. There are no data in Sweden on the actual costs of mastitis,

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nor clinical or subclinical, for Swedish meat- and pelt producing farmers.

Mastitis in sheep can be either clinical or subclinical, bacterial or lentiviral. Most reports on subclinical mastitis (SCM) are from dairy sheep, where the prevalence ranges from less than 10% to 50% or more, see review (Bergonier and Berthelot, 2003). In meat producing herds prevalence on ewe level has been reported to range from 24.1% to 51.2% (Moroni et al., 2007; Arsenault et al., 2008; Zafalon et al., 2016). There are no data on the national prevalence of SCM or IMI in meat- and pelt producing ewes in Sweden, but in a previous pilot study, 24% of the ewes with clinically healthy udders had IMI, mainly caused by coagulase negative staphylococci (CoNS) (Börjesson, 2012).

Subclinical mastitis is an inflammation of the udder without any visible symptoms and is, like clinical mastitis, most often caused by infection with bacteria. Therefore, diagnosis of SCM can be based on inflammatory indicators, measured in milk and additional bacteriology. Milk somatic cell count (SCC) is the most widely used indicator of mastitis in dairy cows, and is also a good predictor of intramammary infection (IMI). Moreover, SCC, especially when measured with a sheep-side test like CMT, is a much cheaper and quicker diagnostic procedure than bacteriology of milk samples. However, the use of SCC as an indicator for mastitis and/or IMI in ewes is not fully evaluated and there is no consensus in the literature about a definite cut-off for SCC that can be used for detection of IMI (Zafalon et al., 2016). Moreover, most studies on SCC as a diagnostic tool for ovine mastitis have been performed in dairy herds and reports from meat and pelt producing herds are scarce. Without any established cut-off for SCC as an indicator of SCM, it is difficult to diagnose SCM or IMI in meat- and pelt producing ewes, and bacteriological culturing of milk must be used instead.

The aim of this study was to investigate the prevalence of IMI and bacterial panorama in ewes with clinically healthy udders, in 22 Swedish meat- and pelt producing herds. Moreover, we wanted to investigate associations between SCC, CMT and IMI, both at weaning and after lambing, and to define cut-offs for SCC and CMT, for detecting udder halves with IMI that would be suitable for use under field conditions.

2. Materials and methods

2.1. Herds and ewes

Twenty-two herds from different parts of Sweden were enrolled in the study. The herds were chosen from a national register provided by the veterinary sheep health services (Gård & Djurhälsan/Farm and Animal Health Service) to get herds from different parts of the country. The median herd size of the 22 participating herds was 120 ewes (50% inter quartile range (IQR): 96–211). Eight of the herds had pure bred ewes (mainly Swedish breeds, only one had pure bred meat breed) and 12 had cross bred ewes (cross between meat breed and Swedish breeds), while two of the herds had both pure bred and cross bred ewes. The ewes were housed for 6.5 months, on average.

2.2. Milk sampling and SCC measurements

Udder half milk samples were collected by veterinarians, veterinary students or experienced farmers per specific instructions. Milk samples were taken after lambing (0–78 days after lambing) and at weaning (one day before, at the day of weaning or one day after) between June 2013 and August 2014. In total, 16 herds were visited twice, once at weaning in 2013 and once after lambing in 2014 (one of these herds was visited at weaning in 2014 and after lambing in 2014). Three herds were visited 3 times, at weaning in 2013, after lambing in 2014 and then again at weaning in 2014. One herd was visited four times, at weaning and after lambing in both 2013 and 2014, and two herds were visited just once after lambing 2014. The plan was to sample the same ewes after lambing and weaning, but this was not possible due to practical reasons. Instead, a convenient sample of 20–25 ewes were selected for milk sampling at each sampling in each herd. Only ewes with normal udder consistency and no visible changes in milk appearance were included in the study (IDF, 2011). For each udder half, the first two or three strips of milk were inspected and discharged, followed by CMT testing using the Scandinavian scoring system (grade 1–5, where 1 is negative and 5 is strong reaction) (Schalm et al., 1971). After cleaning the teat ends with alcohol (70%), an aseptic milk sample (2 ml) was collected from each udder half in sterile test tubes and sent to the National Veterinary Institute, Uppsala, Sweden, for bacteriological analysis and measurement of SCC. Milk aliquots from each test tube were analysed for SCC the same day as sampling, or the day after using the DeLaval Cell Counter (DeLaval International AB, Tumba, Sweden).

2.3. Bacteriological examination of udder half milk samples

Bacteriological culturing of udder half milk samples was performed according to accredited routines (NMC, 1999) (SS-EN ISO/IEC 17025) at the National Veterinary Institute, Uppsala, Sweden. Culturing was performed the same day or the day after the sampling day. Briefly, for each sample 10 µl milk was cultured on a blood (5%) agar plate with esculine, which was incubated at 37 °C for 16–24 h followed by evaluation of growth. The plate was re-evaluated after another 24 h incubation.

An udder half was classified as having IMI if at least one colony-forming unit (CFU) if the SCC was high (CMT 4–5) and growth of relevant mastitis pathogens. If the SCC was lower (CMT 1–3), one colony-forming unit of *Staphylococcus (S.) aureus* or *Streptococcus (Str.) agalactiae* was classified as IMI, and for other bacteria, except for *Corynebacterium* spp., the presence of at least five CFU in pure culture was needed for IMI classification if CMT. For *Corynebacterium* spp., abundant growth ("carpet growth") in pure culture was needed for IMI classification. Samples were classified as contaminated if three or more bacterial species were isolated from one milk sample and growth of a *S. aureus* or *Str. agalactiae* was not identified. If moderate to abundant growth of a relevant udder pathogen was found in combination with a few CFU of several contaminating species the udder half was diagnosed as having an IMI with the relevant udder pathogen.

Bacterial species, except for isolates of *Staphylococcus aureus* with alfa-beta haemolysis, were confirmed by Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF; (Bizzini et al., 2010)). All isolates of staphylococci were examined for betalactamase production by the "clover-leaf" method (Bryan and Godfrey, 1991).

2.4. Statistical analyses

The descriptive statistics were mainly performed using Excel, while the statistical analyses were performed using Stata (Release 13.1; College Station, TX, USA: StataCorp LP).

2.5. Prevalence of IMI and distribution of udder pathogens

The IMI prevalence was calculated at udder half level in total, and separately for samplings at weaning and after lambing. The distribution of different pathogens was summarized at udder half level. The prevalence of IMI was also calculated at ewe level (a ewe was considered to be IMI positive if one or both udder halves had IMI in total and for the two different sampling occasions).

2.6. Associations between SCC, CMT and IMI at udder half level

To investigate the association between udder half SCC (transformed using the natural logarithm to lnSCC) and IMI, as well as between udder half CMT and IMI, multivariable mixed-effect linear regression and

multivariable mixed-effect ordered logistic models were used, respectively. Time of sampling was included as a fix factor in the models. In the models the repeated sampling within ewe and the repeated sampling within herd were included as random effects to account for the fact that repeated samples from the same ewe are more similar than repeated samples from different ewes, and that ewes within a herd are more similar than ewes between different herds. An independent covariance structure was used allowing a distinct variance for each random effect. Two-way interaction between the main effects were tested and kept in the final model if it was significant ($P < 0.05$).

We also investigated if IMI were associated with time of sampling (weaning or lambing) by using an univariable mixed-effect logistic regression model. Repeated sampling within ewe and repeated sampling within herd were included as random effects, and an independent covariance structure was used.

2.7. Optimal cut-offs for SCC and CMT to identify udder halves with IMI

To investigate the optimal cut-off of SCC in order to identify udder halves with IMI we used empirical cut point estimations, using the Youden and "nearest" method (using the cutpt command in Stata). The Youden method gives the cut-off for where the sum of the sensitivity (Se) and specificity (Sp) is maximized, while the nearest method finds the cut-off on the receiver operating characteristic (ROC) curve closest to the point with the perfect Se and Sp (i.e. the point when the 1-Sp = 0 and Se = 1). The optimal SCC cut-off at weaning and after lambing were estimated separately. The results from the Youden and nearest methods were compared and the cut-off with the highest possible Se obtained at the same time as the highest possible Sp was obtained was chosen. A new SCC variable was then created using the optimal cut-off (rounded up to nearest 100,000 cells/ml), and the Se, Sp, positive predictive value (PPV), negative predictive value (NPV) and ROC area ((Se + Sp)/2) for this variable were calculated in order to evaluate the diagnostic properties of SCC, when using the cut-off, to identify udder halves with IMI at weaning and after lambing, respectively.

To investigate the optimal cut-off for CMT three different cut-offs were tested; CMT1 vs. CMT2-CMT5, CMT1-CMT2 vs. CMT3-CMT5 and CMT1-CMT3 vs. CMT4-CMT5, and the Se and Sp was calculated for the different cut-offs at weaning and after lambing. The cut-off with the highest possible Se obtained at the same time as the highest possible Sp was obtained was chosen and for this cut-off the Se, Sp, PPV, NPV and ROC area were calculated to evaluate the diagnostic properties of CMT, when using the chosen cut-off, to identify udder halves with IMI.

2.8. Associations between SCC, CMT and IMI at ewe level

Association between being an ewe with IMI (bacterial findings in one or both udder halves) and having a low or high SCC or CMT score (using the optimal cut-offs on udder half level found above) in none, one or in both udder halves at weaning and after lambing, respectively, were investigated using univariable mixed-effect logistic regression models, including repeated sampling within ewe as well as herd as random factors, using an independent covariance structure.

3. Results

3.1. Prevalence of IMI and distribution of udder pathogens

The overall udder half prevalence was 14% (287 udder halves with IMI of 2101 analysed) and 14% (147 udder halves with IMI of 1054 analysed) at weaning and 13% (140 udder halves with IMI of 1047 analysed) after lambing. Overall, 229 of the 773 ewes had bacterial findings in one or both udder halves at one or more sampling occasions resulting in a IMI prevalence of 30% at ewe level. The IMI prevalence was 25% at ewe level, both at weaning (126 IMI ewes with IMI of 508 sampled) and after lambing (120 ewes with IMI of 533 sampled). At each sampling, the majority (84%) of the 248 ewes with IMI had IMI in one udder half, while 16% had IMI in both udder halves.

Table 1
Bacterial findings in 2101 udder half samples from 773 ewes in 22 herds, presented in total and for samples taken at weaning or after lambing between June 2013 and August 2014.

Bacterial findings	At weaning no. (%)	After lambing no. (%)	Total no. (%)
No growth	796 (76)	762 (73)	1558 (74)
Mixed flora	111 (11)	145 (14)	256 (12)
Coagulase negative staphylococci	85 (8)	80 (8)	165 (8)
Staphylococcus aureus	10 (1)	17 (2)	27 (1)
Mannheimia haemolytica	10 (1)	8 (0.8)	18 (1)
Streptococcus uberis	4 (0.4)	6 (0.6)	10 (0.5)
Streptococcus, other	7 (0.7)	2 (0.1)	9 (0.4)
Bacillus species	9 (0.9)	0 (0)	9 (0.4)
Pantoea species	3 (0.3)	6 (0.6)	9 (0.4)
Escherichia coli	4 (0.4)	3 (0.3)	7 (0.3)
Enterococcus species	1 (0.1)	6 (0.6)	7 (0.3)
Aerococcus viridans	1 (0.1)	4 (0.4)	5 (0.2)
Streptococcus dysgalactiae	1 (0.1)	3 (0.3)	4 (0.2)
Providencia species	2 (0.2)	1 (0.1)	3 (0.1)
Streptococcus pluranimalium	2 (0.2)	1 (0.1)	3 (0.1)
More than one dominating finding	1 (0.1)	1 (0.1)	2 (0.1)
Streptococcus ovis	1 (0.1)	1 (0.1)	2 (0.1)
Streptococcus suis	2 (0.2)	0 (0)	2 (0.1)
Curtobacterium flaccumfaciens pvar poinsettiae	1 (0.1)	0 (0)	1 (0.05)
Gram negative rod bacteria	1 (0.1)	0 (0)	1 (0.05)
Mannheimia glucosida	1 (0.1)	0 (0)	1 (0.05)
Rothia nasimurium	0 (0)	1 (0.1)	1 (0.05)
Streptococcus gallolyticus	1 (0.1)	0 (0)	1 (0.05)
Total	1054	1047	2101

In total, 2134 milk samples from udder halves of 773 ewes were taken for bacteriological analysis, 504 of these ewes were sampled only at weaning or after lambing, 244 were sampled both at weaning and after lambing and 25 were sampled at weaning, after lambing and then at next weaning. In total 534 milk samples were taken at weaning and 533 after lambing.

Bacteriological analyses were only possible in 2101 of the 2134 milk samples (Table 1). Among the 287 milk samples where IMI was identified, CoNS were most prevalent (58%) followed by *S. aureus* (9%) and *Mannheimia (M.) haemolytica* (6%). Among the 165 CoNS findings *S. simulans* was the most common (26%), while *S. warneri* was found in 10%, *S. equorum* in 9%, *S. xylosus* in 8%, and *S. haemolyticus* and *S. chromogenes* in 7% of the CoNS positive samples, respectively (Table 2). In total, 24 (14.5%) of 165 tested CoNS isolates produced betalactamase, while none of the 27 *S. aureus* strains produced β-lactamase.

3.2. SCC, CMT and IMI in udder halves

3.2.1. Descriptive statistics

Information about both SCC and IMI was available for 1582 udder halves from 699 ewes. The median SCC for all milk samples taken at weaning and after lambing were 140,000 cells/ml (50% CR: 59,000–433,000 cells/ml) and 109,000 cells/ml (50% CR: 55,000–297,000 cells/ml), respectively. For IMI positive udder halves the median SCC for milk samples at weaning and after lambing were 1,060,000 cells/ml (50% CR: 174,000–3,929,000 cells/ml) and 955,000 cells/ml (50% CR: 297,000–2,097,000 cells/ml), respectively. For udder halves without bacterial findings the SCC at weaning and after lambing were 117,000 cells/ml (50% CR: 55,000–290,000 cells/ml) and 93,000 cells/ml (50% CR: 53,000–226,000 cells/ml), respectively. The median and mean SCC for different bacterial findings are presented in Table 3. Information about both CMT and IMI was available for 1748 udder halves from 684 ewes. Median CMT score at

Table 2
Coagulase negative staphylococci species findings in 165 udder half samples from 773 ewes in 22 herds, presented in total and for samples taken at weaning or after lambing between June 2013 and August 2014.

Coagulase negative staphylococci	At weaning no. (%)	After lambing no. (%)	Total no. (%)
S. simulans	18 (21)	25 (31)	43 (26)
S. warneri	6 (7)	10 (13)	16 (10)
S. equorum	5 (6)	10 (13)	15 (9)
S. xylosus	12 (14)	1 (1)	13 (8)
S. haemolyticus	7 (8)	5 (6)	12 (7)
S. chromogenes	7 (8)	4 (5)	11 (7)
S. cohnii	2 (2)	4 (5)	6 (4)
S. auricularis	1 (1)	4 (5)	5 (3)
S. rostri	2 (2)	1 (1)	3 (2)
S. pasteuri	1 (1)	1 (1)	2 (1)
S. sciuri	1 (1)	1 (1)	2 (1)
S. caprae	1 (1)	0 (0)	1 (0.6)
S. lentus	1 (1)	0 (0)	1 (0.6)
S. hyicus	0 (0)	1 (1)	1 (0.6)
S. epidermdis	0 (0)	1 (1)	1 (0.6)
S. succinus	0 (0)	1 (1)	1 (0.6)
S. vitulinus	0 (0)	1 (1)	1 (0.6)
Other (CoNS species not determined)	21 (25)	10 (13)	31 (19)
Total	85	80	165

weaning was CMT 2 (50% Central Range (CR): 1–2) and after lambing CMT 1 (50% CR: 1–2). For IMI positive udder halves the median CMT score at weaning and after lambing were CMT 2 (50% CR: 2–4) and CMT 2 (50% CR: 2–4), respectively. For udder halves without bacterial findings the CMT score at weaning and after lambing were both CMT 1 (50% CR: 1–2). For Median CMT scores for different bacterial findings are presented in Table 3.

3.2.2. Associations between SCC, CMT and IMI

There was a significant association between lnSCC and IMI in an interaction with time of sampling; lnSCC was significantly higher for udder halves with IMI compared to udder halves without bacterial findings both at weaning ($P < 0.001$) and after lambing ($P < 0.001$). The lnSCC was significantly higher at weaning than at lambing for udder halves without bacterial findings ($P < 0.001$), but there was no significant difference in lnSCC at weaning and after lambing for udder halves with IMI ($P = 0.95$) (Table 4).

There was a significant association between CMT and IMI in an interaction with time of sampling; CMT was significantly higher for udder halves with IMI compared to udder halves without bacterial findings both at weaning (OR = 6, $P < 0.001$) and after lambing (OR = 14, $P < 0.001$). The CMT was significantly higher at weaning than at lambing for udder halves without bacterial findings (OR = 3, $P < 0.001$), but there was no significant difference in CMT at weaning and after lambing for udder halves with IMI ($P = 0.38$) (Table 4).

3.2.3. Associations between IMI and time of sampling

There was no significant association between IMI and time of sampling ($P = 0.99$) i.e. being sampled at weaning or after lambing did not affect the risk of being IMI positive.

3.2.4. Optimal cut-offs of SCC and CMT for identification of udder halves with IMI

As there was a significant difference in SCC between weaning and after lambing we calculated the optimal cut-offs for identification of udder halves with IMI for each sampling occasion. At weaning, the optimal cut-off for SCC was 513,500 cells/ml (Se = 63%, Sp = 85%), both according to the Youden method and the "nearest" method, and the area under the ROC curve at cut-off was 74%. The optimal cut-off for SCC after lambing was 414,500 cells/ml (Se = 71%, Sp = 87%) according to the Youden method, and 278,500 cells/ml (Se = 76%, Sp = 81%) according to the "nearest" method. In both methods, the area under the ROC curve at cut-off was 79%. For the evaluation of the diagnostic properties of SCC we chose different cut-offs for the SCC at weaning and after lambing. The cut-off at weaning was set to 500,000 cells/ml (based on the results from the Youden and nearest method) and the cut-off after lambing was set to 400,000 cell/ml (based on the results from the Youden method as that gave the highest Sp) (Table 5). The Se and Sp were 63% and 84%, respectively, at weaning, and 71% and 86%, respectively, after lambing. The PPV and NPV were 40% and

Table 3
Distribution of SCC measured with De Laval Cell Counter and CMT scores over bacterial findings in udder halves of ewes. Only bacterial species with at least four observations for one of the measurement are presented. Data for both CMT and SCC was not always available for the same udder half.

Bacterial findings	SCC (x1,000/ml)			CMT	
	n	Mean (SD[a])	Median (50% CR[b])	n	Median (50% CR)
No growth	1231	398 (978)	107 (53; 252)	1319	1 (1; 2)
Mixed flora	153	692 (1633)	105 (54; 241)	198	1 (1; 2)
Coagulase negative staphylococci	122	1374 (1581)	780 (222; 1710)	137	2 (1; 3)
S. simulans	35	1597 (1587)	982 (586; 2137)	34	3 (2; 4)
S. warneri	12	1409 (1201)	1188 (538; 1943)	13	2 (1; 3)
S. chromogenes	10	1712 (1785)	848 (494; 3609)	10	2.5 (2; 3)
S. equorum	8	70 (50)	61 (40; 78)	11	1 (1; 2)
S. haemolyticus	9	2178 (1846)	2176 (250; 3683)	9	3 (1; 5)
S. xylosus	6	80 (54)	74 (46; 92)	13	2 (1; 2)
S. cohnii	5	1684 (2298)	282 (279; 2097)	6	2.5 (1; 3)
S. auricularis	3	406 (301)	327 (152; 739)	4	1.5 (1; 2)
Other (CoNS species not determined)	34	1395 (1634)	743 (201; 1710)	37	3 (1; 4)
Staphylococcus aureus	19	2114 (2060)	1381 (203; 3912)	24	4 (2.5; 5)
Mannheimia spp.	13	3943 (2034)	4216 (2801; 5389)	13	3 (3; 5)
Streptococcus spp.	15	2516 (2583)	1138 (164; 5056)	19	2 (1; 4)
Bacillus spp.	5	1291 (2352)	225 (38; 684)	7	2 (1; 2)
Streptococcus uberis	6	2462 (2423)	1726 (1021; 2210)	5	3 (2; 4)
Enterococcus spp.	5	3242 (2361)	4387 (1322; 4879)	5	4 (2; 4)
Pantoea spp.	4	296 (490)	66 (35; 557)	6	1 (1; 2)
Escherichia coli	5	1956 (2397)	1143 (212; 2452)	4	2 (1.5; 3.5)
Streptococcus dysgalactiae	2	1574 (95)	1574 (1574; 1641)	4	4 (3.5; 4.5)
Other growth	3	801 (857)	376 (240; 1787)	7	2 (2; 5)

[a] SD = standard deviation.
[b] CR = central range.

Table 4
Final result of the multivariable mixed-effect linear regression model investigating associations between SCC (transformed using the natural logarithm) or California Mastitis Test (CMT, scored 1–5) and intramammary infection (IMI). In the model with SCC data from 1430 udder halves of ewes sampled at weaning and after lambing were used and in the model with CMT data from 1550 udder halves were used.

Variable	SCC					CMT				
	β	S.E. (β)	*P*-value	LSM[a]	95% CI[b] (LSM)	β	S.E. (β)	*P*-value	OR[c]	95% CI (OR)
Intercept	4.60	0.14	–	–	–	–	–	–	–	–
Interaction between IMI and time of sampling										
No bacterial finding after lambing	Ref.[d]	–	–	99	76–132	Ref.	–	–	–	–
No bacterial finding at weaning	0.44	0.07	< 0.001	156	119–204	1.09	0.17	< 0.001	2.98	2.15–4.14
IMI at lambing	1.83	0.14	< 0.001	620	433–898	2.65	0.27	< 0.001	14.1	8.25–24.1
IMI at weaning	1.82	0.12	< 0.001	614	441–863	2.94	0.28	< 0.001	18.8	10.9–32.7

[a] LSM = Least square means; x1,000 cells/ml.
[b] CI = confidence interval.
[c] OR = odds ratio.
[d] Ref = reference category.

93%, respectively, at weaning, while the PPV and NPV after lambing were 43% and 95%, respectively. The area under the ROC curve was 74% and 78% at weaning and after lambing, respectively.

As CMT is not a continuous variable the Youden or "nearest" methods were not used to find the optimal cut-off value for CMT to distinguish between udder halves with or without IMI. Instead several cut-offs were tested and the highest Se and Sp in combination (and highest area under ROC curve at cut-off) was obtained using a CMT cut-off at CMT1-CMT2 vs. CMT3-CMT5 (Table 5) both for samples at weaning and after lambing. The Se and Sp using this cut-off were 49% and 82%, respectively, at weaning, and 48% and 88%, respectively, after lambing. The PPV and NPV were 32% and 90%, respectively, at weaning, and 42% and 91%, respectively, after lambing. The ROC area at the cut point were 65% and 68% at weaning and after lambing, respectively.

3.2.5. Associations between SCC, CMT and IMI on ewe level

There was a significant association between being an ewe with IMI (in one or both udder halves) and having a high SCC (i.e. SCC ≥500,000 cells/ml at weaning or SCC ≥400,000 cells/ml after lambing) in one or both udder halves. Ewes with a high SCC in both udder halves had a significantly higher risk of having IMI than ewes with a low SCC (i.e. < 500,000 cells/ml at weaning or < 400,000 cells/ml after lambing) in both udder halves (OR = 4, $P = 0.002$ at weaning, OR = 3.5, $P = 0.01$ after lambing). Moreover, ewes with a high SCC in one udder half and a low SCC in the other udder half had higher risk of having IMI compared to ewes with a low SCC in both udder halves (OR = 7, $P < 0.001$ at weaning, OR = 7, $P < 0.001$ after lambing). There was no significant difference between ewes with high SCC in both udder halves and ewes with a high SCC in one udder half and a low SCC in the other udder half ($P > 0.05$) either at weaning or after lambing.

There was also a significant association between being an ewe with IMI (in one or both udder halves) and having a CMT scores of 1–2 (low CMT scores) or 3–5 (high CMT scores) in one or both udder halves. Ewes with high CMT scores in both udder halves had a significantly higher risk of having IMI than ewes with low CMT scores in both udder halves (OR = 2.5, $P = 0.01$ at weaning, OR = 3, $P = 0.02$ after lambing). Moreover, ewes with a high CMT score in one udder half and low CMT score in the other udder half had higher risk of having IMI compared to ewes with low CMT scores in both udder halves (OR = 10, $P < 0.001$ after weaning, OR = 7, $P < 0.001$ after lambing). Ewes with a high CMT score in one udder half and low CMT score in the other udder half had also a higher risk of having IMI compared with ewes with high CMT scores in both udder halves at weaning (OR = 4, $P = 0.003$), but there was only a tendency for a higher risk after lambing (OR = 2, $P = 0.10$).

4. Discussion

4.1. Prevalence of IMI

In this study, IMI was identified in approximately one third of the meat and pelt-producing ewes with clinically healthy udders. The results are slightly higher than in a Swedish pilot study where 24% of the ewes with clinically healthy udders had IMI (Börjesson, 2012), but very similar to the results of a Canadian study (Arsenault et al., 2008) in meat-producing sheep. In contrast, the prevalence of IMI at ewe level was 51.2% in a study of Italian meat producing sheep (Moroni et al., 2007). The prevalence of IMI at udder half level in the present study, was similar to the results of the previous Swedish study (Börjesson, 2012), but lower than the prevalence in Brazilian and Canadian studies of meat-producing sheep (Arsenault et al., 2008; Zafalon et al., 2016). The reason for the higher prevalence in some other studies is unknown. One could speculate that the relatively good udder health in Swedish ewes is partly due to Swedish sheep herds having good general health. The Swedish herds are rather small and they are sparsely scattered over

Table 5
Numbers of milk samples from udder halves of ewes with a somatic cell count (SCC) above or below the chosen cut-off of 500,000 cells/ml at weaning, above or below the chosen cut-off of 400,000 cells/ml after lambing, or with a CMT (California Mastitis Test) score of 1–2 or 3–5 at lambing or weaning with or without an intramammary infection (IMI). The cut-offs for the SCC was determined by the Youden and "nearest" method to be the optimal cut-off for distinguish between udder halves with or without IMI. CMT score ≥3 was found to be the optimal cut-off for CMT to distinguish between udder halves with or without IMI.

	IMI status	SCC		Total number of milk samples	CMT		Total number of milk samples
At weaning		≥500,000 cells/ml	< 500,000 cells/ml		1–2	3–5	
	IMI	71	42	113	55	57	112
	No growth	105	556	661	114	535	649
	Total	176	598	774	169	592	761
After lambing		≥400,000 cells/ml	< 400,000 cells/ml		1–2	3–5	
	IMI	60	25	85	57	61	118
	No growth	79	492	571	79	592	671
	Total	139	517	656	136	653	789

the country, which gives a favourable situation from a biosecurity point of view.

4.2. Bacterial panorama

The most common IMI found in this study were different species of CoNS which several other studies also have found (Moroni et al., 2007; Arsenault et al., 2008; Zafalon et al., 2016). The distribution of CoNS species in meat-producing ewes varies between studies. (Moroni et al., 2007) found that *S. epidermidis* was the most common CoNS species, while (Clements et al., 2003) found that *S. cohnii* was the most common CoNS, while (Martins et al., 2017) also, as in our study, reports *S. simulans* to be the most common species. Moreover, a review on ovine mastitis (Gelasakis et al., 2015) reported *S. epidermidis* to be the most common CoNS species, followed by *S. chromogenes, S. simulans* and *S. xylosus*; less prevalent species included *S. equorum, S. haemolyticus,* and *S. warneri*. The reason why CoNS are the most important IMI in ewes with clinically healthy udders is not fully understood. In Sweden one reason might the control of *S. aureus* in Swedish sheep herds. The recommendation is to cull all ewes with *S. aureus* IMI and the predominance of CoNS might therefore be a result of a proportional decrease of *S. aureus*. This could also explain the low number of *S. aureus* IMI found in this study.

4.3. Betalactamase production

Almost 15% of the tested CoNS isolates produced betalactamase. This is similar as in a study by (Martins et al., 2017) where 17% of the CoNS isolates where resistant against penicillin.

4.4. SCC and IMI

There were significant associations between elevated SCC, measured with SCC or CMT, and IMI in this study. This is in accordance with previous studies in meat-producing ewes (Arsenault et al., 2008; Zafalon et al., 2016). In the present study, the highest SCC was found in udder halves with findings of *Mannheimia* spp., *S. aureus* or *Enterococcus* spp. In the study of (Moroni et al., 2007), ewes with findings of *S. aureus* had higher SCC than ewes with findings of CoNS, *Bacillus* spp., Gram-negative bacteria or other Gram-positive bacteria. In the study of (Arsenault et al., 2008), a positive association was found between CMT score and findings of CoNS, *M. haemolytica*, *S. aureus* and various *Streptococcus* species.

The optimal cut-off for SCC and CMT for finding udder halves with IMI found in the present study is in agreement with the results from a recent study on meat-producing sheep in Brazil (Zafalon et al., 2016). Clements et al. (2003) did also recommend a cut-off of ≥ 3 for CMT when used as a diagnostic test for IMI in meat-producing ewes. However, they also recommended a SCC cut-off of > 1,200,000 cells/ml when used as a diagnostic test for IMI, especially when IMI prevalence is low, which differs from the results of the present study and that of (Zafalon et al., 2016). In the study of (Clements et al., 2003), only 60 udder halves were investigated bacteriologically and using CMT and automated SCC, which could be one explanation to the diverging SCC results.

The Se and Sp of SCC and CMT in correct classifying a ewe with IMI, using the optimal cut-off to obtain the highest possible Se at the same time as obtaining the highest possible Sp, was in the present study general low. This could be an indication that SCC and CMT might not be the optimal diagnostic tool to use for ewes to correct distinguish between IMI positive or bacteriological negative udder halves. Other cut-off values could be used to increase e.g. Se to increase the performance in detecting truly IMI positive udder halves, but that would reduce the Sp i.e. reducing the performance in detecting truly bacteriologically negative udder halves. However, the NPV was around 90% for all the cut-offs chosen so by using SCC or CMT as a diagnostic tool 90% of the udder halves that is classified as negative will be truly IMI negative. In contrast, only 30–50% of the udder halves classified as IMI positive using SCC or CMT will be truly IMI positive. A better way for finding ewes with IMI than just using the cut-offs seems to be to compare the SCC or CMT scores for the two udder halves. If one or both udder halves had high SCC or high CMT scores the risk of being IMI positive was much higher (especially when just one udder half had high SCC or CMT) than if both udder halves had low SCC or low CMT scores. Sheep owners should therefore be particularly observant when they find a difference in CMT score between udder halves of an ewe, and it may be appropriate to take a milk sample from the udder half with the highest score for bacteriological analysis.

5. Conclusions

One third of Swedish meet and pelt producing ewes without clinical signs in the udder had IMI in one or both udder halves at one or several sampling occasions. The SCC and CMT was significantly elevated in udder halves with IMI. Among udder halves with IMI, CoNS was the most common bacterial finding. A CMT score of ≥ 3 at weaning or lambing, and a SCC of $\geq 500{,}000$ cells/ml at weaning or $\geq 400{,}000$ cells/ml after lambing gave the highest possible Se at the same time as the highest possible Sp in correct classifying udder halves as having IMI or not. Moreover, a difference in CMT score between udder halves was a good indicator of IMI. In the field, farmers can be recommended to use CMT for udder health screening and to collect milk samples from ewes with high CMT scores in one or both udder halves.

Conflict of interest

None.

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