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Risk factors for intramammary infection in meat- and pelt producing ewes with clinically healthy udders

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ABSTRACT

Mastitis is prevalent both in milk- and meat- and pelt producing sheep flocks and is an important disease with substantial effects on economy, animal welfare and antibiotic use, and in dairy flocks also on milk quality and safety. In meat- and pelt producing flocks, the farmers are mainly concerned by clinical mastitis (CM), and most cases of subclinical mastitis (SCM) are not detected and not dealt with, posing a risk for poor udder health in the flock. Studies on risk factors for SCM and/or intramammary infections (IMI) in meat producing flocks are scarce and specific risk factors for weaning and lambing are absent. The aim of this study was therefore to investigate associations between risk factors, at ewe and flock level, for IMI after lambing and at weaning in Swedish meat- and pelt producing ewes with clinically healthy udders. Twenty-two meat- and pelt producing flocks from different parts of Sweden were enrolled in this cross-sectional study. Udder half milk samples were collected at weaning and after lambing from ewes with clinically healthy udders, for bacteriological investigations. Data on ewe and flock level risk factors were recorded. In total, 753 ewes were sampled at least once, and the overall IMI prevalence was 22.5 %. Older ewes, ewes with three or more lambs, ewes that were hard to milk when collecting a milk sample, ewes in moderate size flocks, in flocks with hay as a bedding material and in flocks with less cases of CM had higher risk of IMI. Most of the risk factors associated with IMI in this study differed between weaning and lambing. This study has provided us with novel knowledge on how different factors influence udder health of meat- and pelt producing ewes.

1. Introduction

Mastitis is prevalent both in milk- and in meat- and pelt producing sheep flocks (Bergonier et al., 2003). It is the most common reason for culling of ewes, and costs for replacement of animals and treatments are of substantial economic importance (Aitken, 2007). Most studies on ovine mastitis have been conducted in dairy flocks (Lianou and Fthenakis, 2020), dominating the sheep industry in many countries, while few studies have been performed in other production types. Differences between these production systems are likely to be associated with some differences in mastitis risk factors. In Sweden, sheep are mainly kept for meat- and pelt production, and to a lesser extent for dairy or wool. In meat- and pelt producing flocks (also called suckler flocks), the farmers are mainly concerned by clinical mastitis (CM), but there are reasons to believe that also subclinical mastitis (SCM) is of importance for the flock health and economy. Subclinical mastitis in ewes causes decreased milk production (Fthenakis and Jones, 1990; Marti De Olives et al., 2013), which can lead to decreased growth rates and increased mortality in lambs (Gross et al., 1978; Fthenakis and Jones, 1990; Moroni et al., 2007; Arsenault et al., 2008; Huntley et al., 2012; McLaren et al., 2018). There is also an association between CM in suckler ewes and a previous history of SCM caused by the same pathogen (Watkins et al., 1991). Intramammary infection (IMI) is the main cause of both clinical and subclinical ovine mastitis. In a previous study, it was found that IMI, mainly due to staphylococci, was found in 30 % of the ewes and in 14 % of the udder half milk samples in Swedish clinically healthy meat- and

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pelt producing ewes (Persson et al., 2017).

Studies on risk factors for SCM and/or IMI in meat producing sheep flocks are more scarce than in dairy sheep (Lianou and Fthenakis, 2020). However, as the age of the ewe, breed, body condition score (BCS), udder conformation, previous history of CM, number of suckling lambs, season, region and high stocking density have been associated with the risk for SCM and/or IMI in meat producing flocks where milk has been collected at different occasions, from lambing to weaning depending on study (Gross et al., 1978; Nilsson, 1984; Watkins et al., 1991; Sevi et al., 1999; Arsenault et al., 2008; Zafalon et al., 2016; McLaren et al., 2018).

In the design and implementation of a control program for IMI, different risk factors should be addressed for the specific farm and period (Gelasakis et al., 2015). Lambing and weaning are two important periods of the sheep year in Sweden, but knowledge on specific risk factors for each of these periods is absent (Lianou and Fthenakis, 2020).

The aim of this study was to investigate associations between potential risk factors, at ewe and flock level, and IMI after lambing and at weaning in Swedish meat- and pelt producing ewes with clinically healthy udders.

2. Materials and methods

This study is part of a larger project from which data on prevalence of IMI, pathogens, somatic cell counts (SCC) and California mastitis test (CMT) scores in ewes with clinically healthy udders, in meat- and pelt producing flocks in Sweden, using the same material as in this study, was presented in a previous publication (Persson et al., 2017). In the present study, the data on IMI was used to study potential risk factors for such infections.

2.1. Flocks and ewes

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

2.2. Milk sampling and bacteriological examination of milk samples

Udder half milk samples were collected by veterinarians, veterinary students, or experienced farmers per specific instructions. Milk samples were taken once at weaning (one day before, at the day of weaning or one day after) and once after lambing (0–78 days after lambing) between June 2013 and August 2014 (two flocks were not sampled at weaning). In total, 15 flocks were visited twice, once at weaning in 2013 and once after lambing in 2014, and 1 flock was visited once after lambing in 2014 and once at weaning in 2014. Three flocks were visited 3 times, at weaning in 2013, after lambing in 2014 and then again at weaning in 2014. One flock was visited four times, after lambing and at weaning in both 2013 and 2014, and two flocks were visited just once after lambing 2014. A convenience sample, where the first 20–25 ewes encountered were chosen, was selected for milk sampling at each sampling in each flock. Only ewes with normal udder consistency and no visible changes in milk appearance were included in the study. For more details on individual milk sampling for bacteriology, CMT and SCC, see (Persson et al., 2017).

Bacteriological culturing of udder half milk samples was performed according to accredited routines (SS-EN ISO/IEC 17025) at the National Veterinary Institute, Uppsala, Sweden. For more details on bacteriological examination and classification, see (Persson et al., 2017).

2.3. Registration on ewe and flock data

At each flock visit, the person collecting the milk samples also recorded data on breed, age, lambing number, lambing date, number of lambs, lambing difficulties, and cases of CM in the previous lactation for each sampled ewe. The same person also recorded the BCS for each ewe using a scale from 1 to 5 where 1 indicates skinny and 5 obese (Sjödin, 2007). Udder characteristics, i.e., hard to milk when collecting a milk sample and presence of blind teats and teat injuries, were also recorded. See Table 1 for more details.

Each farmer provided flock data by answering a web-based questionnaire (Easy research/Questback, Stockholm) containing 45 questions on flock size, production system, housing, feeding, bedding, pasture, routines at lambing and at weaning, prevalence, and management of CM etc. See Table 1 for more details.

2.4. Statistical analyses

All data was transferred to Excel and then exported to Stata (Release 13.1; College Station, TX, USA: StataCorp LP) where all statistical analyses were performed. For the statistical analyses, the bacteriological results from 753 ewes with a total of 1040 samplings (as some of the ewes were sampled both at weaning and lambing) were included. All except one of the 22 participating flocks answered the web-based questionnaire. However, two of the flocks had ewes that were only sampled at lambing, and not all farmers answered all questions, hence, the number of observations included in the analyses varied depending on question analysed.

2.5. Associations between risk factors and being an ewe with IMI

Associations between being a ewe with IMI in one or both udder halves (the dependent variable) and ewe and flock factors that was registered at the visits and in the questionnaire (the independent variables) were investigated using univariable and multivariable mixed-

Table 1
Final multivariable logistic regression model of associations between ewe and flock factors and udder health status (intramammary infection) of ewes at weaning in 22 flocks visited during June 2013 to August 2014 (n = 434 ewes, pseudo R^2 = 0.13).

	β[1]	SE	OR[2]	95% CI (OR)	*P*-value
Intercept	1.88	0.70			
Age					
One	−1.64	1.03	0.19	0.03; 1.46	0.11
Two	−2.19	0.66	0.11	0.03; 0.41	0.001
Three	−2.30	0.62	0.10	0.03; 0.34	<0.001
Four	−1.39	0.59	0.25	0.08; 0.79	0.02
Five	−1.35	0.60	0.26	0.08; 0.84	0.02
Six	−1.07	0.61	0.34	0.10; 1.13	0.08
Seven	−0.92	0.67	0.40	0.11; 1.48	0.17
≥Eight	Referent				
Number of lambs					
None	0.18	1.73	1.20	0.04; 35.8	0.92
One	−1.26	0.41	0.28	0.12; 0.64	0.002
Two	−0.85	0.33	0.43	0.22; 0.82	0.01
Three to four	Referent				
Udder half difficult to milk					
None	Referent				
Yes, one or both	1.75	0.46	5.74	2.33; 14.1	<0.001
Bedding material					
Straw	−1.08	0.42	0.34	0.15; 0.77	0.01
Straw and sawdust	−1.52	0.76	0.22	0.05; 0.97	0.04
Hay	Referent				

[1] β = regression coefficient.
[2] OR = Odds Ratio.

effect logistic regression models. As the milk samplings were done at weaning and at lambing, two separate models for each sampling were made; one investigating factors associated with being a ewe with IMI at weaning and one investigating factors associated with being a ewe with IMI at lambing. All factors investigated were used in both models. Flock was included in the models as random factor and an independent covariance structure was used. The independent variables are presented in Supplementary Table 1. Variables having an association with a *P*-value ≤0.20 in the univariable analyses and with less than 10 % missing values were included in the multivariable analyses (one for IMI at weaning and one for IMI after lambing). Collinearity between the independent variables was assessed pairwise by Spearman rank correlations. When proof of collinearity (r ≥0.70) existed, the variable with the lowest *P*-value in the univariable analysis was selected. A manual stepwise backward variable selection procedure was used in the multivariable analyses where the initial model included all independent variables as main effects. Two-way interactions were then investigated between all main effects. To remain in the final model, the variable, or interaction, had to have a *P*-value ≤0.05. If the random effect was not significant in the final model, a conventional logistic regression model was used. The model fit of the multivariable analyses was tested by Hosmer-Lemeshow goodness-of-fit test and by visual examination of diagnostic plots according to (Dohoo et al., 2010).

3. Results

3.1. Descriptive statistics

In total, 753 ewes were sampled at least once and 225 of them had IMI in one or both udder halves at one or more sampling occasion resulting in an overall IMI prevalence of 22.5 %. The IMI prevalence was 22.5 % at weaning and 21.9 % after lambing. A full description of the bacterial findings, as well as CMT-scores and SCC, is given in (Persson et al., 2017). The distribution of ewes with or without IMI over ewe and flock factors are presented in Supplementary Table 1.

The overall median flock prevalence of ewes with IMI was 19.8 % (CR: 13.6–29.2 %); 22.5 % (CR: 13.4–27.7 %) at weaning and 20.0 % (CR: 12.0–27.3 %) after lambing. The flock prevalence varied markedly both between flocks (from 0% to 58 %) and within flocks between samplings (from 0 % to 44 %) (Fig. 1).

3.2. Associations between risk factors and IMI at weaning

The results of the univariable analysis of associations between ewe and flock factors and IMI at weaning are presented in Supplementary Table 1. In total, 15 factors were significantly ($P \leq 0.20$) associated with IMI at weaning in the univariable analysis. The correlation between parity and age, and between flock size and number of lambing ewes in the flock was >0.70, hence, only one of these factors, respectively, could be included in the multivariable model. As age and flock size had a lower *P*-value than parity and number of lambing ewes in the flock, respectively, when looking at the associations with IMI at weaning and after lambing, these factors were chosen to be included in the multivariable model. The results of the final multivariable analysis of associations between IMI at weaning and ewe and flock factors are presented in Table 1. As the random effect of flock was not significant in the final model a conventional logistic regression model was used. In this analysis 4 variables, i. e. age, number of delivered lambs per ewe, having one or two udder halves hard to milk when collecting a milk sample and bedding material, remained significant ($P < 0.05$). The odds ratio of IMI at weaning was significantly lower in ewes two to five years old compared to ewes eight years old or older, while there was no significant difference in odds ratio between ewes one, six or seven years old compared to ewes eight years old or older. The odds ratio of IMI at weaning was significantly lower in ewes that had one or two lambs compared to ewes that had three or four lambs, while there was no significant difference in odds ratio between ewes that did not have any lambs at weaning and those that had three to four lambs. Ewes with one or two udder halves that were hard to milk when collecting a milk sample had a higher odds ratio of IMI at weaning than ewes where it was easy to milk both udder halves. Moreover, the odds ratio of IMI at weaning was lower for ewes in flocks where straw or straw and shavings were used as bedding material compared to ewes in flocks where hay was used as bedding material. No significant two-way interactions were found and, moreover, the random effect of flock was not significant, hence, an ordinary logistic regression model was used, where flock as random factor was not included, in the final model.

3.3. Associations between risk factors and IMI after lambing

In total, 16 factors were significantly associated with IMI after lambing with a $P \leq 0.20$ in the univariable analysis (Supplementary Table 1). The results of the final multivariable analysis of associations

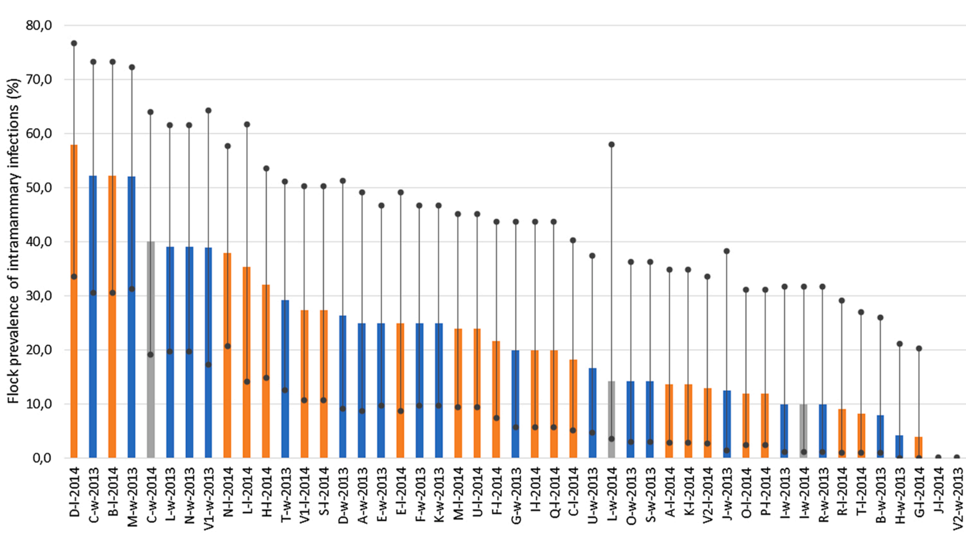

Fig. 1. Flock prevalence and confidence interval of intramammary infection in ewes in 22 flocks (A to V) at weaning (w; blue (2013) or orange (2014) bars) or after lambing (l; grey bar (2014)) between June 2013 and August 2014. In flock V they had both spring (V1) and winter lambings (V2).

between IMI after lambing and ewe and flock factors are presented in Table 2. As the random effect of flock was not significant in the final model a conventional logistic regression model was used. In this analysis 3 factors, flock size, age and number of CM cases during 2014, were significantly associated with the odds ratio of being a ewe with IMI after lambing. The odds ratio of IMI after lambing was higher in flocks with a flock size of >160 ewes compared to in flocks with a flock size ≤160 ewes. There were no significant differences in odds ratio of IMI after lambing between flocks with a flock size of ≤100 or 101–160 ewes (P = 0.06). The odds ratio of IMI after lambing was lower for ewes 2, 3 and 6 years old compared to ewes 5 years old. The odds ratio of IMI after lambing was lower in flocks with five or more CM cases during 2014 than in flocks with less than five CM cases during 2014.

4. Discussion

This is the first study on risk factors for IMI in meat- and pelt producing sheep in Sweden. Intramammary infections were common, and several risk factors were identified. Age of the ewe, number of lambs and udders hard to milk when collecting a milk sample were ewe level risk factors for IMI. Number of cases of CM previous year, bedding material and flock size were flock level risk factors for IMI. Only age was a risk factor for IMI both at lambing and after weaning.

The prevalence of IMI have already been presented and discussed in a previous paper (Persson et al., 2017). In brief, IMI was identified in approximately one third of the meat and pelt-producing ewes with clinically healthy udders that were included in the study, and non-aureus staphylococci were the most common pathogens.

Table 2
Final multivariable logistic regression model of associations between ewe and flock factors and udder health status (intramammary infection) of ewes after lambing in 22 flocks visited during June 2013 to August 2014 (n = 495 ewes, pseudo R^2 = 0.08).

	β[1]	SE	OR[2]	95% CI (OR)	*P*-value
Intercept	0.43	0.77			
Age					
One	−1.14	0.72	0.32	0.08; 1.33	0.12
Two	−1.12	0.38	0.32	0.15; 0.68	0.003
Three	−0.89	0.35	0.41	0.21; 0.81	0.01
Four	−0.46	0.33	0.63	0.32; 1.23	0.18
Five	Referent				
Six	−1.15	0.48	0.31	0.12; 0.80	0.02
Seven	−0.37	0.48	0.69	0.27; 1.78	0.45
≥Eight	−0.11	0.59	0.90	0.28; 2.86	0.86
Flock size					
≤100	−1.26	0.31	0.28	0.15; 0.52	<0.001
101–160	−0.72	0.29	0.49	0.27; 0.86	0.01
≥161	Referent				
Number of clinical mastitis cases in the year of participation					
<5	Referent				
≥5	−1.01	0.26	0.36	0.22; 0.60	<0.001

[1] β = regression coefficient.
[2] OR = Odds Ratio.

4.1. Associations between risk factors at ewe level and IMI at weaning or after lambing

In this study, old ewes had a higher risk for IMI at weaning than younger ewes and ewes with fewer lambs. Younger ewes had lower odds ratio for IMI at weaning compared to older ewes. The association between age and IMI was not as clear when looking at IMI after lambing, although the trend was similar with higher incidences in older ewes. Age of the ewe (Gross et al., 1978; Nilsson, 1984; Watkins et al., 1991; Arsenault et al., 2008) has been described as a risk factor for SCM or IMI in previous studies where milk has been collected at different occasions, from lambing to weaning, in meat producing flocks, but was not supported by (Murphy et al., 2018). A study on dairy sheep (Lafi et al., 1998) suggests that the udder becomes more susceptible, probably because of a cumulative stress on mammary tissue after several lactations or due to increased prevalence of infection and permanent glandular damage from previous infections.

Ewes with three or more lambs had a higher risk for IMI at weaning than younger ewes and ewes with fewer lambs. Number of suckling lambs (Gross et al., 1978; Nilsson, 1984) have been described as risk factor for SCM or IMI and also for CM (Larsgard and Vaabenoe, 1993) in previous studies in meat producing flocks but this was not supported by (Murphy et al., 2018). Damage to the teats by sucking have been suggested as a possible cause of increased risk of SCM in dairy sheep (Lafi et al., 1998) and CM in meat sheep (Waage and Vatn, 2008). Another possible explanation is an increased risk of teat contamination (Arsenault et al., 2008) with increased litter size. The presence of *Staphylococcus aureus* in the nose of lambs, and transfer of these pathogens to the dam in meat sheep flocks has been suggested (Mørk et al., 2012), as well as transmission of *Mannheimia haemolytica* from the tonsils of lambs to the teats of the ewes (Fragkou et al., 2011).

Ewes in this study that were hard to milk when collecting a milk sample had higher risk for IMI at weaning. This has not been described elsewhere and we have no good explanation for the finding. The prevalence of ewes with this condition did not differ between sampling occasions and at each sampling, at least one ewe with one hard to milk udder half was found. It is possible that the described phenomenon could lead to rough suckling that might predispose to IMI. Only ewes with healthy udders and milk were included in the study, and" hard to milk" was not an exclusion criterion. Still, we could speculate that this might be a symptom of mastitis. Another reason for this finding could be stress among the ewes during sampling.

We found no significant association between breed or BCS and risk for IMI, which is partly in line with (Murphy et al., 2018), but contradictory to other studies where a low BCS was associated with CMT-positive udder halves (Arsenault et al., 2008) and where breed was associated with SCM (Nilsson, 1984; Zafalon et al., 2016) and CM (Larsgard and Vaabenoe, 1993) in meat ewes. Most other studies had other breeds than in our study, where mainly cross breeds were included, why it is difficult to make a comparison. One explanation to the lack of association between BCS and IMI at weaning could be that in the present study only few ewes had a low BCS.

4.2. Associations between risk factors at flock level and IMI at weaning or at lambing

Flock size was associated with IMI in this study and in the multivariable analysis there was a lower risk for IMI in ewes after lambing in flocks with smaller flock size compared to flocks with more ewes. This finding was in line with those of (Nilsson, 1984) who reported a tendency that flocks with smaller group size (<20 ewes/group) had less SCM cases than flocks with larger group size, but overall, smaller flocks were included in this study. To our knowledge, no other studies on meat- or pelt producing sheep have investigated this trait. A high animal density (Sevi et al., 1999) and a smaller airspace (Sevi et al., 2001) have been associated with a higher risk for SCM in ewes, but this was not per se investigated in our study.

Bedding material was found to be a risk factor for IMI at weaning with a lower risk in flocks where straw or straw and shavings were used as bedding material compared to flocks where hay was used as bedding material. The reason for this is not clear and this finding may be spurious as it was only one flock that used hay as bedding material. However, as the statistical model was on ewe level, i.e., comparing ewes with IMI, not flocks with ewes with IMI, while adjusting for similarities within flock by including flock as random factor, the results seem valid although hard to explain. Moreover, the use of hay was surprising as hay is not recommended as bedding material in Sweden. Poor litter management has been associated with elevated SCC in dairy ewes (Sevi et al., 2003) but there are to our knowledge no studies on how different bedding materials affect udder health in ewes.

The risk for IMI after lambing was significantly lower in flocks with more CM cases during 2014 than in flocks with fewer cases. This was a surprising finding and opposite to that of (Watkins et al., 1991). In the latter study, an association between the development of CM and previous SCM caused by the same organism was found, which supports the theory that there is an interlinkage between SCM and CM. In the present study, we did not have any data on CM causing pathogens. (Arsenault et al., 2008) saw an association between previous CM and increased risk of CMT-positivity, but not to an increased risk for IMI. They suggest that antibiotic treatments and/or natural healing were generally effective enough to eliminate or reduce the bacterial charge to an undetectable level. Since CM often treated with antibiotics in Sweden, this could partly also explain our findings.

4.3. Differences between risk factors at weaning and lambing

The risk factors associated with IMI in this study differed between weaning and lambing, indicating that different preventive measures might be of more importance at weaning than at lambing, and vice versa. This view is also supported by (Gross et al., 1978) who found differences in associations between age of the ewe and number of lambs, and milk CMT at lambing compared to 3–7 weeks after lambing. Ewe age, breed, and number of lambs did not affect direct SCC measured either at <5 days or 30–35 days in milk (Murphy et al., 2018). To our knowledge, no other studies have explored the association between different risk factors at weaning and lambing. (Watkins et al., 1991) collected milk samples throughout a lactation from lambing to weaning but did not compare different risk factors with days in milk. Age was the only factor that was associated with IMI at both weaning and lambing, although the correlation was not completely clear at lambing. Number of lambs, hard milked udders and hay as bedding material were risk factors associated with a higher risk of IMI at weaning. Flock size and previous history of CM were risk factors associated with IMI at lambing. It is difficult to explain the reasons behind the differences between weaning and lambing and more knowledge about risk factors at different time points during production are needed to make specific recommendations how to prevent IMI in meat producing ewes.

4.4. Methodological considerations

In this study we used convenience sampling of both farms and ewes, hence, our results may not be a representative sample of Swedish meat sheep producing flocks or of ewes within each flock. Many associations were tested in this study and, hence, the results should be interpreted with caution as there can be an increased risk for type I errors, i.e., to find significant results even though there are no true associations. Therefore, some of the associations found might be just due to chance. Since it is impossible to know which of these associations could be due to chance, additional studies are needed to further confirm the findings in this study.

5. Conclusion

Older ewes, ewes with three or more lambs, ewes that were hard to milk when collecting a milk sample, ewes in moderate size flocks, in flocks with hay as a bedding material and in flocks with less cases of CM had higher risk of IMI. Most of the risk factors associated with IMI in this study differed between weaning and lambing. This study has provided us with novel knowledge on how different factors influence udder health of clinically healthy meat and pelt producing ewes.

Data availability

No data was used for the research described in the article.
Data will be made available on request.
The data that has been used is confidential.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.smallrumres.2021.106595.

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