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Relationships among intramammary health, udder and teat characteristics, and productivity of extensively managed ewes

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Abstract

Mastitis is an economically important disease and its subclinical state is difficult to diagnose, which makes mitigation more challenging. The objectives of this study were to screen clinically healthy ewes in order to 1) identify cultivable microbial species in milk, 2) evaluate somatic cell count (**SCC**) thresholds associated with intramammary infection, and 3) estimate relationships between udder and teat morphometric traits, SCC, and ewe productivity. Milk was collected from two flocks in early (<5 d) and peak (30 to 45 d) lactation to quantify SCC (n = 530) and numerate cultivable microbial species by culture-based isolation followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (**MALDI-TOF MS**; n = 243) identification. Within flock and lactation stage, 11% to 74% (mean = 36%) of samples were culture positive. More than 50 unique identifications were classified by MALDI-TOF MS analysis, and *Bacillus licheniformis* (18% to 27%), *Micrococcus flavus* (25%), *Bacillus amyloliquefaciens* (7% to 18%), and *Staphylococcus epidermidis* (26%) were among the most common within flock and across lactation stage. Optimum SCC thresholds to identify culture-positive samples ranged from 175 × 10^3 to 1,675 × 10^3 cells/mL. Ewe productivity was assessed as total 120-d adjusted litter weight (**LW120**) and analyzed within flock with breed, parity, year, and the linear covariate of $\log_{10}$ SCC (**LSCC**) at early or peak lactation. Although dependent on lactation stage and year, each 1-unit increase in LSCC (e.g., an increase in SCC from 100 × 10^3 to 1,000 × 10^3 cells/mL) was predicted to decrease LW120 between 9.5 and 16.1 kg when significant. Udder and teat traits included udder circumference, teat length, teat placement, and degree of separation of the udder halves. Correlations between traits were generally low to moderate within and across lactation stage and most were not consistently predictive of ewe LSCC. Overall, the frequencies of bacteria-positive milk samples indicated that subclinical mastitis (**SCM**) is common in these flocks and can impact ewe productivity. Therefore, future research is warranted to investigate pathways and timing of microbial invasion, genomic regions associated with susceptibility, and husbandry to mitigate the impact of SCM in extensively managed ewes.

Key words: etiology, ewe productivity, mastitis, sheep, somatic cell count, udder morphology

Abbreviations

BCS	body condition score
DS	degree of separation of the udder halves
LSCC	log_{10}-transformed somatic cell count
LWW	litter weaning weight
LW120	total 120-d adjusted litter weight
MALDI-TOF MS	matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry
MSU	Montana State University
SCC	somatic cell count
SCM	subclinical mastitis
SDA	Sabouraud dextrose agar
TC	terminal composite
TL	teat length
TP	teat placement
TSA	trypticase soy agar
UC	udder circumference
USD	United State dollar
USSES	USDA U.S. Sheep Experiment Station
$USSES_M$	multiparous USSES ewes
$USSES_P$	primiparous USSES ewes
UWS	udder wool score
YI	Youden's Index

Introduction

Economic losses attributed to clinical mastitis in sheep can be substantial and include increased ewe turnover, increased animal health costs, and reduced lamb survival and growth. Conington et al. (2008) estimated that clinical mastitis costs the UK purebred Texel industry alone over $3.5 USD million annually. Similar economic analyses have not been conducted in the U.S. sheep industry, but udder health-related issues, including hard-bag syndrome and clinical mastitis, account for nearly 14% of ewes culled each year (USDA APHIS Sheep, 2012). Signs of clinical mastitis are somewhat easy to identify and therapeutically intervene, but subclinical infection presents no visible clinical signs in the infected animal. Still, ewes with subclinical mastitis (**SCM**) have an intramammary infection that can be detected through screening milk samples for causative bacteria or inferred by quantifying somatic cell count (**SCC**; Clements et al., 2003; Świderek et al., 2016).

It has been estimated that the prevalence of SCM (16.7% to 30.0%; Maisi et al., 1987; Arsenault et al., 2008; Persson et al., 2017) is far greater than clinical mastitis (0% to 8.1%; Arsenault et al., 2008; Koop et al., 2010) in nondairy flocks. However, associations between dam SCM status and lamb performance can be difficult to estimate from field data. As such, the effect of SCM on ewe and lamb performance has ranged from insignificant (Hueston, 1980; Kirk et al., 1980; Keisler et al., 1992) to substantial (Gross et al., 1978; Ahmad et al., 1992). However, collecting milk samples to detect or infer SCM in a commercial setting is cost-prohibitive and impractical. Recent work has reported associations between udder morphometry and health in nondairy ewes (Cooper et al., 2013; McLaren et al., 2018; Crump et al., 2019) and may serve as a more practical selection tool to reduce mastitis. Therefore, the objectives of this study were to 1) identify cultivable microbial species in milk, 2) evaluate SCC thresholds associated with intramammary infection, and 3) estimate relationships between udder and teat morphometric traits, SCC, and ewe productivity in extensively managed, range-type ewes.

Materials and Methods

All animal handling protocols were approved by the Montana State University Agricultural Animal Care and Use Committee (2017-AA04) and the Institutional Animal Care and Use Committee of the U.S. Sheep Experiment Station (1803).

Animal management

Two flocks of ewes in semi-extensive management systems were used in this study, one located at Montana State University's (**MSU**) Red Bluff Research Ranch (Norris, MT; 45.6°N 111.7°W) and the second at the USDA U.S. Sheep Experiment Station (**USSES**; Dubois, ID; 44°10′N 112°13′W). Mean annual minimum temperature (MSU = 2 °C and USSES = –0.6 °C), maximum temperature (14.6 and 12.9 °C), snowfall (1,372 and 1,212 mm), and precipitation (445 and 302 mm) are similar between the two sites (Western Regional Climate Center; MSU = site 246,157 and USSES = site 102,707). However, several aspects of husbandry differ between the flocks, which may contribute to variation in health and performance.

Ewes at MSU lambed in drylot during April and May and, within 1 h of parturition, were moved indoors to individual bonding pens with their lamb(s) for 12 to 24 h. Ewes and lambs were then transitioned through incrementally larger groups in mixing pens for the next 7 d. During this time, ewes were fed chopped grass (brome, garrison, and orchard) and alfalfa hay. Ewes and lambs were then placed in larger paddocks until turnout to summer range at 30 to 45 d post-lambing. Sheep were then herded as one contiguous band until weaning.

Ewes at USSES were lambed in March and April and managed similarly to those at MSU except they and their lambs were housed in individual pens for 36 to 48 h. Furthermore, USSES ewes remained in drylot before turnout where they were fed a total mixed ration (45% alfalfa hay, 20% whole corn, 20% sugar beet pulp, 10% barley straw hay, and 5% sugar beet condensed separator byproduct) with an added coccidiostat until turnout to summer grazing (30 to 45 d postpartum). At turnout, USSES sheep were allocated to one of the two bands and grazed sagebrush steppe and subalpine forest until weaning.

Ewes at both MSU and USSES were sampled from their larger flocks described above. Two- to 5-yr-old Targhee (n = 45) and Rambouillet ewes (n = 29) at MSU were identified while in mixing pens, approximately 3 to 5 d after parturition and in a manner that roughly balanced for ewe age and number of nursing lambs. Apart from a brief separation during milk collection, sampled MSU ewes were managed alongside the entire flock. Two separate groups of USSES ewes were sampled and are differentiated in this study as multiparous and primiparous. Both multiparous and primiparous USSES ewes (**$USSES_M$** and **$USSES_P$**, respectively) were identified for study inclusion before parturition and were managed separately from one another and the remaining flock until turnout. The multiparous group was sampled in 2017 and 2018 and contained 2- to 7-yr-old Suffolk (n = 93) and terminal composite (**TC** = three-eight Suffolk, three-eight Columbia, and one-quarter Texel; n = 51) ewes. The primiparous group was sampled in 2018 and contained 1-yr-old Columbia (n = 8), Suffolk (n = 12), and TC (n = 13) ewes.

Milk collection

All MSU and USSES ewes were free of an active infection of clinical mastitis at the time of milk collection. Ewes were separated from their lamb(s) for ~30 min, administered ½ mL of oxytocin intramuscularly, and restrained on a milking stand. Teats were then disinfected with 99% isopropyl alcohol, two streams of residual milk were discarded, and ewes were manually milked using aseptic techniques (gloves, sterile tubes, etc.). The timing and frequency of milk sample collection and whether samples were from individual halves or the whole udder were different between MSU, $USSES_M$, and $USSES_P$ ewes. Milk samples were collected from MSU and $USSES_P$ ewes shortly after lambing (3 to 5 d; early lactation) and before turnout to summer grazing (35 to 45 d; peak lactation). $USSES_M$ ewes were collected at peak lactation only. Milk samples were obtained separately from each udder-half for MSU and $USSES_M$ ewes and composited equally between halves for $USSES_P$ ewes. For microbial testing, raw milk (5 mL) was transferred to sterile conical tubes and stored at −25 °C. For SCC testing, 35 mL of milk was collected, preserved with 8 mg Bronopol and 0.3 mg Natamycin (Microtabs II; D & F Control Systems, Inc.; Dublin, CA), and then refrigerated until testing (<72 h).

Milk SCC and microbial culturing

SCC was quantified in duplicate on all samples within 72 h of collection using a LactiCyte HD (Page & Pedersen International, Ltd.; Hopkinton, MA) somatic cell counter and replicates were averaged for each sample. Bacteria isolation procedures were conducted on a proportion of frozen milk samples (n = 186) from MSU ewes and all samples (n = 57) from $USSES_P$ ewes. Before doing so, milk was slowly thawed at room temperature, and then 1 mL was pipetted into a microtube and centrifuged at 5,000 × g for 5 min to separate fat, supernatant liquid, and milk pellet. Fat and most of the supernatant liquid were discarded, and the pellet was resuspended in the remaining supernatant by vortex. A 10-µL inoculating loop was used to streak the resuspended pellet onto compartmentalized plates (Thermo Fischer Scientific Inc.; Waltham, MA) containing one each of four microbiological growth media: MacConkey agar (HiMedia Laboratories Pvt. Ltd.; Mumbai, India), trypticase soy agar (**TSA**; Becton, Dickinson and Company; Sparks, MD), TSA-5% sheep blood agar (Hardy Diagnostics; Santa Maria, CA), and Sabouraud dextrose agar (**SDA**; Neogen Corporation; Lansing, MI). These media were chosen to selectively cultivate different microbes: MacConkey agar isolates Gram-negative and enteric bacilli, TSA supports the growth of fastidious and non-fastidious microorganisms, TSA-5% sheep blood contains added nutrients for microbial growth compared with TSA, and SDA cultivates fungi. Plates were incubated aerobically at 37 °C for 18 to 24 h. The number of colonies was quantified on culture-positive plates and colony morphologies were recorded. Plates that exhibited no growth were re-incubated for an additional 24 h.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification

Culture-positive samples were further subjected to identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (**MALDI-TOF MS**). The direct colony transfer method was used by transferring purified colonies in triplicate with a sterile 1-µL inoculating loop onto a 48-well steel-target plate. Then, 1 µL of Matrix A CHCA (alpha-cyano-4-hydroxycinnamic acid) was added to the center of each well. Mass spectrometry-based identifications were conducted using an Agena MassARRAY Instrument (Agena Bioscience, San Diego, CA) and a spectrum match factor library. If no replicate resulted in a successful match, samples were reanalyzed in triplicate.

Multiplex PCR methods

Milk samples from MSU (n = 175) and $USSES_P$ (n = 55) ewes that were of sufficient volume for DNA extraction following culture methods were further evaluated by multiplex polymerase chain reaction (PCR). There are currently no commercial PCR test kits that are specific to SCM in sheep, but there are many similarities between cases of SCM in sheep and cattle. The Thermo Scientific PathoProof Complete-16 kit (Waltham, MA) was utilized with the Primer Mix 4 for Applied Biosystems 7500 and 7500 Fast instruments. This test kit identifies 16 possible taxa commonly isolated from cases of bovine intramammary infection, including *Corynebacterium bovis*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella oxytoca* and/or *Klebsiella pneumoniae*, *Mycoplasma bovis*, *Mycoplasma* spp., *Prototheca* spp., *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus* spp., *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Trueperella pyogenes*, and/or *Peptoniphilus indolicus*, and many common yeasts. Additionally, the kit screens for the β-lactamase penicillin resistance gene in staphylococci, including *Staphylococcus aureus* and all major coagulase-negative staphylococci. Four PCR reactions were run for each milk sample (Eurofins DQCI, LLC.; Mounds View, MN).

Conformation traits and ewe productivity

At the time of milk sampling, all MSU and $USSES_M$ ewes had udder and teat morphometric traits collected, which are described in Table 1. Objective traits included udder circumference (**UC**) and average teat length (**TL**). Teat placement (**TP**) and degree of separation (**DS**) of udder halves were both assessed on a 9-point scale adapted from Casu et al. (2006). A scale was developed to assess the degree of wool covering on the udder (udder wool score [**UWS**]). Body condition score (**BCS**) was also assessed at this time and expressed on a 1 (very thin) to 5 (obese) scale.

Lambs in both flocks were weighed within 12 h of birth and at weaning, which was used to calculate 120-d adjusted body weight. Total litter weaning weight (**LWW**) was the sum of the ewe's 120-d adjusted litter body weight and was calculated for MSU and $USSES_M$ ewes. Lambs that died or were transferred to the nursery were not included in their dam's LWW. Therefore,

Table 1. Description of udder and teat morphometric, health, and anatomical traits collected from MSU and USDA $USSES_M$ ewes

Trait	Units	Description
TL	cm	Average teat length; measured from base to distal end.
UC	cm	Udder circumference; measured around udder at widest point.
TP	1 to 9	Teat placement; adapted from Casu et al. (2006)
DS	1 to 9	Degree of separation of udder halves; adapted from Casu et al. (2006).
UWS	1 to 3	Udder wool score (1 = little or no wool coverage, 2 = moderate wool coverage, and 3 = heavy wool coverage).
BCS	1 to 5	Body condition score assessed by palpating the spinous and transverse processes for degree of subcutaneous adipose tissue.

LWW evaluates maternal productivity and is a composite trait that reflects the joint effects of ewe maternal ability and lamb sex, survival, and growth to weaning.

Data analyses

Culture, MALDI-TOF MS, and PCR results for MSU and $USSES_P$ samples were analyzed within year, stage of lactation, and flock. Binomial proportions and 95% confidence intervals of taxa identified within culture- or PCR-positive samples were estimated using the *binom* package of R (Dorai-Raj, 2014; R Core Team, 2019). SCCs were log_{10} transformed (**LSCC**), and the TTEST procedure of SAS (v. 9.4; SAS Inst. Inc., Cary, NC) was used to compare means between culture- or PCR-status groups (positive or negative). SCC thresholds were then analyzed from 100 × 10^3 to 2,000 × 10^3 cells/mL (every 25 × 10^3 cells/mL) and the ability of each to predict culture or PCR status was assessed. In the context of the present study, sensitivity is the proportion of positive samples predicted to be positive based upon their SCC at a given threshold, while specificity is the proportion of negative samples predicted to be negative based upon their SCC at a given threshold. Youden's Index (**YI**) then attempts to optimize sensitivity and specificity (YI = sensitivity + specificity – 100%).

For all other analyses involving MSU and $USSES_M$ ewes, udder-half SCCs greater than 2,000 × 10^3 cells/mL were removed (MSU = 15; $USSES_M$ = 39). A total of 281 and 249 udder-half LSCC records from 74 MSU and 144 $USSES_M$ ewes, respectively, remained. The CORR procedure was used to estimate Pearson correlation coefficients between udder-half LSCC within and across lactation time point. LWW was analyzed separately for MSU and $USSES_M$ ewes using the GLM procedure. Classification effects included ewe age (2 or 3+ yr), breed (MSU = Rambouillet or Targhee; $USSES_M$ = Suffolk or TC), and year (2017 or 2018). Since individual udder-half LSCC within lactation period was highly correlated, the maximum udder-half LSCC was fit as a linear covariate for MSU (early and mid-lactation) and $USSES_M$ (mid-lactation) LWW. Equality of LSCC slopes between classification effect levels and all two-way interactions among classification effects were tested and removed if not significant.

Pearson correlation coefficients between udder and teat morphometric traits within and across lactation time point were estimated for MSU and $USSES_M$ ewes. The GLM procedure was then used to evaluate factors affecting maximum udder-half LSCC in early and mid-lactation for MSU and mid-lactation for $USSES_M$ ewes. Main classification effects included ewe age, breed, year, number of lambs born (1 or 2+), and UWS (low, medium, or high), while TL, UC, TP, DS, and BCS were averaged across lactation stage (MSU only) and fit as linear covariates.

Results

Frequency of identified taxa—MALDI-TOF MS

Across all milk samples collected, 36% were culture positive and 52 taxa were identified by MALDI-TOF MS. Taxa identified at a frequency ≥10% within positive samples during each lactation period are displayed in Tables 2 and 3. Within flock and year in early lactation (Table 2), isolates from 9% to 20% of culture-positive samples were unable to be identified by MALDI-TOF-MS. Of the most frequent species identified, only *Bacillus amyloliquefaciens* and *Bacillus licheniformis* were present in both flocks and years at early lactation. Within the MSU flock in 2017, *Bacillus altitudinis* and *B. licheniformis* were the most prevalent. During 2018, MSU cultures most often comprised *B. altitudinis*, *B. amyloliquefaciens*, and *Bacillus subtilis*. The most common species identified by MALDI-TOF MS within culture-positive $USSES_P$ samples were *Staphylococcus epidermidis*, *B. licheniformis*, and *Enterococcus faecium*.

The proportion of culture-positive samples was numerically lower at peak than early lactation for all flocks and years (Table 3). The frequency of unidentified culture-positive samples was similar between early and peak lactation for $USSES_P$ ewes but greater at peak lactation for MSU. Of the most frequent species identified, none were common to both flocks and years during peak lactation. Only one sample was identified at MSU in 2017 (*Micrococcus flavus*) and no species were identified at a frequency greater than 10% at MSU in 2018. As in early lactation, *B. licheniformis* was prevalent at peak lactation within $USSES_P$ samples, while *Bacillus* species and several staphylococci were identified at lower frequencies.

Frequency of identified taxa—multiplex PCR

Across all milk samples collected, 14% were PCR positive and 12 of the 16 tested taxa were identified by multiplex PCR and those identified at a frequency ≥5% within positive samples

Table 2. Number of samples collected in early lactation (<5 d) within year and flock and estimated species frequency (95% confidence interval) within culture-positive samples determined by culture/MALDI-TOF MS methods

	Flock (year)		
Item	MSU (2017) ewes	MSU (2018) ewes	$USSES_P$ ewes
No. of samples[1]	49	50	31
No. of positive, %	15 (30.6)	17 (34.0)	23 (74.2)
Species[2]			
Unidentified[3]	0.20 [0.04, 0.48]	0.12 [0.01, 0.36]	0.09 [0.01, 0.28]
Bacillus altitudinis	0.27 [0.08, 0.55]	0.18 [0.04, 0.43]	—
Bacillus amyloliquefaciens	0.07 [0.00, 0.32]	0.18 [0.04, 0.43]	0.04 [0.00, 0.22]
Bacillus licheniformis	0.13 [0.02, 0.4]	0.12 [0.01, 0.36]	0.22 [0.07, 0.44]
Bacillus subtilis	—	0.18 [0.04, 0.43]	0.04 [0.00, 0.22]
Enterococcus faecium	—	—	0.17 [0.05, 0.39]
Staphylococcus auricularis	—	—	0.13 [0.03, 0.34]
Staphylococcus epidermidis	—	0.06 [0.00, 0.29]	0.26 [0.10, 0.48]
Staphylococcus lugdunensis	—	0.12 [0.01, 0.36]	—

[1]MSU samples considered on an udder-half basis, and $USSES_P$ samples considered on a whole-udder basis.
[2]Only species that occurred at a frequency ≥ 0.10 within a flock (year) are presented.
[3]Unidentified, samples that were culture positive but not identified by MALDI-TOF MS.

during each lactation period are displayed in Tables 4 and 5. No 2017 MSU samples were positive for the PCR test screen in early lactation. Within the MSU flock during 2018 at early lactation, only four samples were PCR positive, of which *Staphylococcus* spp. were identified in two and *E. coli*, *T. pyogenes* and/or *P. indolicus*, and yeasts identified in one each. The frequency of PCR positives in early lactation was greatest for USSES$_P$ samples, and *E. coli* was the most often identified, while other taxa were only found in one sample each.

The frequency of PCR-positive samples increased slightly in all flocks and years from early to peak lactation (Table 5). Only one sample was PCR positive in peak lactation at MSU in 2017 (yeasts). The taxa most commonly identified in the five PCR-positive 2018 MSU samples during peak lactation were *E. coli*, *Staphylococcus* spp., and *S. aureus* along with the staphylococcal β-lactamase gene. The frequency of PCR positives was again greatest for USSES$_P$ samples at peak lactation, and *Klebsiella* spp., *E. coli*, and *Staphylococcus* spp. were the most common.

SCC thresholds to infer infection status

Mean LSCC between culture/MALDI-TOF MS or PCR-status groups was compared within flock/year and lactation period only when ≥5 positive cases were detected, and results are displayed in Table 6. In general, LSCC of positive samples was numerically greater than negative samples. However, LSCC was not significantly affected by the overall culture status (culture negative vs. culture positive; $P \geq 0.33$), MALDI-TOF MS identification status (0 taxa identified vs. ≥ 1 taxa identified; $P \geq 0.28$), overall PCR status (0 taxa identified vs. ≥ 1 taxa identified; $P \geq 0.17$), or any taxa-specific status ($P \geq 0.22$) at either lactation period within any flock/year.

Despite this, SCC thresholds were evaluated for their ability to detect culture and multiplex PCR status within flock/year and lactation period. Calculated sensitivity and specificity corresponding to the SCC that maximized YI are displayed in Table 7. Optimum thresholds were dependent upon flock/year and whether culture/MALDI-TOF MS or PCR status was being evaluated but ranged from 175 × 10^3 to 1,675 × 10^3 cells/mL and

Table 3. Number of samples collected in mid-lactation (30 to 45 d) within year and flock and estimated species frequency (95% confidence interval) within culture-positive samples determined by culture/MALDI-TOF MS methods

	Flock (year)		
Item	MSU (2017) ewes	MSU (2018) ewes	USSES$_P$ ewes
No. of samples[1]	37	50	26
No. of positive, %	4 (10.8)	12 (24.0)	16 (61.5)
Species[2]			
Unidentified[3]	0.75 [0.19, 0.99]	0.33 [0.10, 0.65]	0.12 [0.02, 0.38]
Bacillus licheniformis	—	—	0.25 [0.07, 0.52]
Bacillus spp.	—	—	0.12 [0.02, 0.38]
Micrococcus flavus	0.25 [0.01, 0.81]	—	—
Staphylococcus aureus	—	—	0.12 [0.02, 0.38]
Staphylococcus auricularis	—	—	0.12 [0.02, 0.38]
Staphylococcus lugdunensis	—	—	0.12 [0.02, 0.38]

[1]MSU samples considered on an udder-half basis, and USSES$_P$ samples considered on a whole-udder basis.
[2]Only species that occurred at a frequency ≥ 0.10 within a flock (year) are presented.
[3]Unidentified, samples that were culture positive but not identified by MALDI-TOF MS.

Table 4. Number of samples collected in early lactation (<5 d) within year and flock and estimated species frequency (95% confidence interval) within positive samples determined by PCR methods

	Flock (year)		
Item	MSU (2017) ewes	MSU (2018) ewes	USSES$_P$ ewes
No. of samples[1]	45	47	29
No. of positive, %	0	4 (8.5)	10 (34.5)
Species[2]			
Enterococcus spp.	—	—	0.10 [0.00, 0.45]
Escherichia coli	—	0.25 [0.01, 0.81]	0.60 [0.26, 0.88]
Klebsiella spp.	—	—	0.10 [0.00, 0.45]
Prototheca spp.	—	—	0.10 [0.00, 0.45]
Trueperella pygoenes and/or *Peptoniphilus indolicus*	—	0.25 [0.01, 0.81]	0.10 [0.00, 0.45]
Staphylococcus aureus	—	—	0.10 [0.00, 0.45]
Staphylococcus spp.	—	0.50 [0.07, 0.93]	—
Streptococcus dysgalactiae	—	—	0.10 [0.00, 0.45]
Streptococcus uberis	—	—	0.10 [0.00, 0.45]
Yeasts	—	0.25 [0.01, 0.81]	0.10 [0.00, 0.45]

[1]MSU samples considered on an udder-half basis, and USSES$_P$ samples considered on a whole-udder basis.
[2]Only species that occurred at a frequency ≥ 0.05 within a flock (year) are presented.

Table 5. Number of samples collected in mid-lactation (30 to 45 d) within year and flock and estimated species frequency (95% confidence interval) within positive samples determined by PCR methods

	Flock (year)		
Item	MSU ewes (2017)	MSU ewes (2018)	$USSES_P$ ewes
No. of samples[1]	34	49	26
No. of positive, %	1 (3.0)	5 (10.2)	13 (50.0)
Species[2]			
Enterococcus spp.	—	—	0.08 [0.00, 0.36]
Escherichia coli	—	0.60 [0.15, 0.95]	0.31 [0.09, 0.61]
Klebsiella spp.	—	—	0.54 [0.25, 0.81]
Trueperella pygoenes and/or *Peptoniphilus indolicus*	—	—	0.08 [0.00, 0.36]
Staphylococcal β-lactamase gene	—	0.20 [0.01, 0.72]	—
Staphylococcus aureus	—	0.20 [0.01, 0.72]	0.08 [0.00, 0.36]
Staphylococcus spp.	—	0.60 [0.15, 0.95]	0.23 [0.05, 0.54]
Streptococcus agalactiae	—	0.20 [0.01, 0.72]	—
Streptococcus dysgalactiae	—	—	0.08 [0.00, 0.36]
Yeasts	1.00 [0.03, 1.00]	—	—

[1]MSU samples considered on an udder-half basis, and $USSES_P$ samples considered on a whole-udder basis.
[2]Only species that occurred at a frequency ≥ 0.05 within a flock (year) are presented.

Table 6. Mean (± SE) LSCC of culture- and taxa-status groups (NEG, negative; POS, positive) for the most frequent taxa identified by culture/MALDI-TOF MS and PCR methods within flock/year and lactation stage

	Flock (year)					
	MSU (2017)		MSU (2018)		$USSES_P$	
Method/species[1]	NEG	POS	NEG	POS	NEG	POS
Culture[E]	5.35 ± 0.07	5.41 ± 0.12	5.71 ± 0.08	5.59 ± 0.10	5.85 ± 0.18	5.90 ± 0.11
S. epidermidis[E]	—	—	—	—	5.87 ± 0.11	5.97 ± 0.05
PCR[E]	—	—	—	—	5.90 ± 0.12	5.86 ± 0.10
E. coli[E]	—	—	—	—	5.76 ± 0.09	5.98 ± 0.08
Culture[M]	—	—	5.73 ± 0.06	5.84 ± 0.12	6.37 ± 0.09	6.34 ± 0.14
PCR[M]	—	—	5.73 ± 0.06	5.98 ± 0.15	6.30 ± 0.16	6.41 ± 0.10
Klebsiella spp.[M]	—	—	—	—	6.34 ± 0.12	6.40 ± 0.12

[1]Culture, overall culture status subsequent taxa identified by MALDI-TOF MS; PCR, overall PCR status subsequent taxa identified by PCR.
[E]Samples taken in early lactation (<5 d).
[M]Samples taken in mid-lactation (30 to 45 d).

large variations in sensitivity (12% to 100%) and specificity (12% to 91%) were observed.

Effect of ewe SCC on productivity

Estimated Pearson correlation coefficients between udder-half LSCC within and across lactation period for MSU ewes are displayed in Table 8. The correlation between udder-half LSCC in early lactation was moderate in 2017 but strong in 2018 ($P < 0.01$) and mid-lactation udder-half LSCC was strongly correlated in both years ($P < 0.01$). However, correlation coefficients between the same or opposite udder-half LSCC across lactation period were not significantly different from zero ($P \geq 0.31$). Within $USSES_M$ ewes, the correlation between left and right udder-half LSCC at mid-lactation was moderate in 2017 (0.39; $P < 0.01$) but strong in 2018 (0.82; $P < 0.01$).

Least-squares means for the main classification effects and solutions for the linear covariates of LSCC on LWW for MSU and $USSES_M$ ewes are displayed in Table 9. Within the MSU flock, LWW was greater in 2017 than 2018 ($P < 0.01$), greater for 3+ than 2-yr-old ewes ($P < 0.01$), and not different between Rambouillet and Targhee ewes ($P = 0.43$). The slope of early lactation LSCC on LWW was dependent on year ($P = 0.01$) and was significant and negative in 2017 ($P = 0.01$) but not significantly different from zero in 2018 ($P = 0.10$). However, mid-lactation LSCC did not affect LWW in MSU ewes ($P = 0.25$). Within $USSES_M$ ewes, LWW was greater in 2018 than 2017 ($P < 0.01$), greater for 3+ than 2-yr-old ewes ($P < 0.01$), and greater for TC than Suffolk ewes ($P < 0.01$). The slope of mid-lactation LSCC on LWW was also dependent on year for $USSES_M$ ewes ($P < 0.01$) and was significant and negative in 2017 ($P < 0.01$) but not significantly different from zero in 2018 ($P = 0.08$).

Udder and teat conformation traits and ewe SCC

Estimated Pearson correlation coefficients between udder and teat conformation traits within and across lactation stage for MSU and $USSES_M$ ewes are displayed in Table 10. Within lactation period in both flocks, correlations between traits were generally low to moderate (–0.25 to 0.36) except for UC and TP at mid-lactation in MSU ewes, which were more strongly and negatively correlated (–0.53). Within traits for MSU ewes, TL, UC, TP, and DS were moderately or strongly correlated (0.47 to 0.63) between early and mid-lactation. Estimated correlation coefficients between traits across lactation stage were generally low to moderate (–0.30 to 0.33) for MSU ewes.

Early lactation LSCC for MSU ewes was greater in 2018 than 2017 (5.82 ± 0.12 vs. 5.36 ± 0.11; $P = 0.03$), but no other model

Table 7. Sensitivity (Sen, %) and specificity (Spe, %) at the SCC threshold corresponding to maximum YI (SCC_{MaxYI}, cells/mL) to predict culture and taxa status within flock/year and lactation stage

	Flock (year)								
	MSU (2017)			MSU (2018)			$USSES_P$		
Method/species[1]	SCC_{MaxYI}	Sen	Spe	SCC_{MaxYI}	Sen	Spe	SCC_{MaxYI}	Sen	Spe
Culture[E]	275 × 10^3	53	68	168 × 10^4	12	91	175 × 10^3	87	12
S. epidermidis[E]	—	—	—	—	—	—	625 × 10^3	83	40
PCR[E]	—	—	—	—	—	—	275 × 10^3	80	32
E. coli[E]	—	—	—	—	—	—	350 × 10^3	100	39
Culture[M]	—	—	—	475 × 10^3	75	47	900 × 10^3	75	20
PCR[M]	—	—	—	375 × 10^3	100	39	350 × 10^3	100	15
Klebsiella spp.[M]	—	—	—	—	—	—	158 × 10^4	86	32

[1]Culture, overall culture status (no growth, growth); PCR, taxa identified by PCR (0 taxa, ≥ 1 taxa).
[E]Samples taken in early lactation (<5 d).
[M]Samples taken in mid-lactation (30 to 45 d).

Table 8. Estimated Pearson correlation coefficients between udder-half LSCC of milk samples collected from MSU ewes at early and mid-lactation in 2017 and 2018

Year	Trait[1,2,3]	$LSCC_{L,E}$	$LSCC_{R,M}$	$LSCC_{L,M}$
2017	$LSCC_{R,E}$	0.42*	0.08	0.08
	$LSCC_{L,E}$	—	0.16	0.10
	$LSCC_{R,M}$	—	—	0.93*
2018	$LSCC_{R,E}$	0.84*	−0.12	−0.07
	$LSCC_{L,E}$	—	0.02	0.11
	$LSCC_{R,M}$	—	—	0.86*

[1]$LSCC_E$, LSCC collected at early lactation (<5 d).
[2]$LSCC_M$, LSCC collected at mid-lactation (30 to 45 d).
[3]R and L, right and left half of the udder.
*Estimated correlation coefficient is different from zero ($P < 0.01$).

Table 9. Least-squares means (±SE) of main effects and solutions for the linear covariate of LSCC on LW120 for sampled ewes

		Flock	
Effect	Level	MSU, kg	$USSES_M$, kg
Year	2017	38.8 ± 1.44[a]	52.9 ± 2.57[b]
	2018	35.8 ± 1.44[b]	59.3 ± 2.90[a]
Age	2	31.9 ± 1.74[b]	50.8 ± 3.36[b]
	3+	42.7 ± 1.40[a]	61.4 ± 2.00[a]
Breed[1]	R/S	38.1 ± 1.74	49.5 ± 2.28[b]
	T/TC	36.4 ± 1.40	62.6 ± 2.93[a]
$LSCC_E$[2]	2017	−9.54 ± 3.66*	—
	2018	7.29 ± 4.43	
$LSCC_M$[3]	2017	2.87 ± 2.49	−16.1 ± 5.23*

[1]R (Rambouillet) and T (Targhee) ewes were sampled at MSU; S (Suffolk) and TC were sampled at $USSES_M$.
[2]$LSCC_E$, LSCC collected at early lactation (<5 d); its effect on MSU ewe performance was dependent on year ($P = 0.01$).
[3]$LSCC_M$, LSCC collected at mid-lactation (30 to 45 d); its effect on $USSES_M$ ewe performance was dependent on year ($P < 0.01$).
[a,b]Means within a column and effect with no common superscript are different ($P < 0.01$).
*The solution for the linear covariate of LSCC is different from zero ($P < 0.01$).

effect was significant at early ($P \geq 0.24$) or mid-lactation ($P \geq 0.10$) in this flock. Year also affected LSCC during mid-lactation in $USSES_M$ ewes and was greater in 2018 than 2017 (5.73 ± 0.10 vs. 5.28 ± 0.09; $P < 0.01$). Additionally, the linear covariate for DS was significant and indicated a positive relationship with mid-lactation LSCC (0.07 ± 0.03; $P = 0.05$) within this flock.

Discussion

Etiology of ovine SCM

Overall, the frequency of culture-positive milk samples across flocks in the present study (11 to 74%) was generally greater than past research has reported. Microbial detection was less frequently achieved with PCR compared with the culture-based identifications, likely because the multiplex PCR test kit was developed for cattle and had a limited number of microbial targets included in screening panel. In clinically healthy meat-type ewes, culture-positive milk samples have been reported to be between 6% and 19% (Maisi et al., 1987; Watson et al., 1990; Watkins et al., 1991; Arsenault et al., 2008; Persson et al., 2017). In investigations of SCM in U.S. sheep populations specifically, frequencies ranged between 11% and 27% (Ahmad et al., 1992; Keisler et al., 1992).

Reports of *Mannheimia* spp. isolation in subclinically infected nondairy ewes have varied between <5% and 23% (Watkins et al., 1991; Clements et al., 2003; Hariharan et al., 2004; Arsenault et al., 2008); however, *Mannheimia* spp. were not detected in the present study. *Staphylococcus aureus* is a commonly isolated bacterium in mastitis cases in nondairy ewes, with frequencies between 45% and 65% in clinical cases (Mørk et al., 2007; Koop et al., 2010), but frequencies have ranged considerably in subclinical cases (40% in Watson et al., 1990; 9% in Arsenault et al., 2008). In the present study, *S. aureus* was identified at a low frequency (<8%), which agrees with Arsenault et al. (2008).

Streptococcus spp. have also been isolated from 5% of clinically infected ewes (Mørk et al., 2007; Blagitz et al., 2014) and 2% to 42% in ewes with SCM (Watkins et al., 1991; Arsenault et al., 2008). Of the *Streptococcus* spp. isolated, *S. agalactiae*, *S. uberis*, and *S. bovis* are common (Ariznabarreta et al., 2002); however, MALDI-TOF MS only identified *S. mitis* and *S. suis* at low levels in the present study (<2%). *Escherichia coli* has been commonly reported as present in ewe milk (4% to 14%; Watkins et al., 1991; Lafi et al., 1998; Mørk et al., 2007) and was the most frequently identified taxon by PCR in the present study (up to 26%).

Bacillus spp. were generally the most frequently isolated bacteria in the present study (0% to 30%, mean = 16.5%). However, since members of this genus are present in the

Table 10. Estimated Pearson correlation coefficients between udder and teat conformation traits within and across lactation stage for sampled ewes

Flock	Trait[1]	UC_E	TP_E	DS_E	TL_M	UC_M	TP_M	DS_M
MSU	TL_E	0.27*	0.17	0.28*	0.51*	−0.04	0.23*	0.04
	UC_E	—	−0.13	0.17	−0.04	0.63*	−0.17	0.22
	TP_E	—	—	−0.06	0.33*	−0.30	0.51*	0.02
	DS_E	—	—	—	0.03	0.07	0.11	0.47*
	TL_M	—	—	—	—	−0.18	0.29*	−0.05
	UC_M	—	—	—	—	—	−0.53*	0.08
	TP_M	—	—	—	—	—	—	0.23*
$USSES_M$	TL_M	—	—	—	—	−0.03	0.36*	−0.25*
	UC_M	—	—	—	—	—	−0.09	0.18*
	TP_M	—	—	—	—	—	—	−0.22*

[1]Full description of traits (TL, UC, TP, DS, UWS, and BCS) is provided in Table 1.
[E]Trait measured at early lactation (<5 d).
[M]Trait measured at mid-lactation (30 to 45 d).
*Estimated Pearson correlation coefficient is different from zero ($P \leq 0.05$).

environment and soil, Clements et al. (2003) attributed detection to potentially contaminated samples or culture media. Nevertheless, others have reported frequencies between 2.1% and 6.4% in subclinically infected ewes (Watkins et al., 1991; Al-Majali and Jawabreh, 2003; Arsenault et al., 2008; Spanu et al., 2011). Moreover, metagenomics techniques have also identified *Bacillus* spp. as a component of the milk and teat microbiomes in subclinically infected dairy cows (Woodward et al., 1988; Bhatt et al., 2012; Braem et al., 2013; Bonsaglia et al., 2017), thus providing a connection between the environment–teat–milk microbiomes and support for *Bacillus* spp. and other microbial species commonly found in the environment as mastitis-causing agents.

More than 50 bacterial taxa were identified using MALDI-TOF MS. While this diagnostic tool has been widely adopted for use in clinical microbiology, the technique is novel in some applications and has not been widely adopted in sheep research. Still, several studies have validated and utilized this technology to identify pathogens isolated in subclinical and clinical mastitis in dairy cows (Barreiro et al., 2010; Werner et al., 2012). In the present study, a variable proportion of culture-positive samples could not be confidently classified by MALDI-TOF MS, which is typically a limitation of library-based identification methods.

SCC thresholds to infer SCM

Past research in dairy ewes has not agreed on a narrow SCC range to infer SCM, with suggested thresholds between 300 × 10^3 and 1,139 × 10^3 cells/mL (González-Rodríguez et al., 1995; Suarez et al., 2002; Riggio et al., 2013). Fewer studies have investigated SCC thresholds that indicate intramammary infection status in meat and wool type ewes but results range between 205 × 10^3 and 1,660 × 10^3 cells/mL (Maisi et al., 1987; Fthenakis et al., 1991; Clements et al., 2003; Świderek et al., 2016). In the present study, YI was maximized between 175 × 10^3 and 1,675 × 10^3 cells/mL and varied by flock, lactation stage, and pathogen identification. Still, these thresholds fall within range of those suggested by previous researchers.

Relationship between SCC and ewe productivity

Researchers have reported that naturally occurring (Torres-Hernandez and Hohenboken, 1979; McCarthy et al., 1988) and experimentally induced SCM (Fthenakis and Jones, 1990) reduced meat and wool type ewe milk yields by between 12% and 58%. Reduced milk quantity and quality would be expected to have large impacts on lamb survival and growth. Gross et al. (1978) reported lambs reared by dams with bilateral SCM weighed between 4.5 and 7.6 kg less at weaning compared with lambs reared by healthy ewes. In a Midwestern farm flock, Ahmad et al. (1992) reported that the effect of dam SCM on lamb BW gain varied in statistical significance by study year (4% to 10%). Finally, Huntley et al. (2012) estimated that lambs reared by dams with an SCC greater than 400 × 10^3 cells/mL weighed 0.73 kg less at 10 wk than lambs reared by dams with an SCC below this threshold. Still, others have reported no relationship between ewe SCC and lamb growth (Hueston, 1980; Keisler et al., 1992; McLaren et al., 2018).

The results from the present study indicated that the effect of SCC on ewe productivity was dependent on production year. In 2017, MSU ewes with an SCC of 500 × 10^3 cells/mL in early lactation were expected to wean 4.8 kg less lamb than ewes with an SCC of 100 × 10^3 cells/mL. Similarly, $USSES_M$ ewes with an SCC of 500 × 10^3 cells/mL during mid-lactation in 2017 were expected to wean 8 kg less lamb than ewes with an SCC of 100 × 10^3 cells/mL. This equates to over $19 USD and $32 USD in lost lamb revenue based on average market prices during data analysis of the present study ($4.01 USD/kg; USDA AMS, 2020) per ewe, respectively. Therefore, reducing ewe udder inflammation and/or the incidence of SCM in commercial sheep flocks could have significant and positive economic benefits.

Udder morphometry and SCC

Several scoring systems have been developed for dairy sheep to evaluate udder and teat characteristics and estimate their relationship with milk production and intramammary health. Casu et al. (2006) estimated that most of the udder and teat traits were not strongly genetically correlated with milk yield (−0.10 to 0.15) except for udder depth (−0.48), but indicators of SCM were not included in their analyses. Fernández et al. (1997) estimated negligible phenotypic correlations between SCC and udder depth (0.13) and teat size (0.18). Finally, Legarra and Ugarte (2005) estimated a moderate genetic correlation between milk yield and udder depth (0.43) but low correlations between SCC and udder attachment (−0.27) and teat size (0.29).

Albeit the heritability of clinical mastitis was low (0.09), estimates were greater for TP (0.35), udder depth (0.21), DS (0.27), and TL (0.42) in Texel flocks (Crump et al., 2019). McLaren et al. (2018) also evaluated Texels and reported that the heritability of SCC at ~4 wk postpartum was low (0.11) but its genetic correlation

was moderate for udder depth (0.61), udder length (0.53), teat angle (–0.41), and teat width (0.44). However, at 11 wk postpartum, genetic correlations between SCC and udder morphometric traits were insignificant or low. While average TL was associated with LSCC in early lactation for MSU ewes and average DS was associated with LSCC in mid-lactation for $USSES_M$ ewes, most of the udder and teat measurements did not influence LSCC in either flock.

Conclusions

The present research is unique from previous investigations in that extensively managed ewes were sampled across two flocks in the intermountain west, whereas similar studies in the United States have been limited to a single flock. Abundant microbial species potentially involved in the pathogenesis of mastitis were frequently isolated in milk from healthy-appearing ewes. It was also determined that milk SCC can be used to infer intramammary infection status in extensively managed ewes but thresholds were variable, and phenotyping is cost- and labor-prohibitive at the producer level. Therefore, more easily measured indicator traits could be useful selection criteria for reducing mastitis. While significant correlations were detected among many udder and teat conformation traits, most were not consistently predictive of ewe SCC. Additional studies investigating effective methods (e.g., selection, environmental management, and nutritional) of reducing mastitis in extensively managed ewes are warranted.

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Conflict of interest statement

No authors have any commercial or financial interests that could be perceived as a conflict of interest.

Literature Cited

Ahmad, G., L. L. Timms, D. G. Morrical, and P. O. Brackelsberg. 1992. Dynamics and significance of ovine subclinical intramammary infections and their effects on lamb performance. *Sheep Res. J.* **8**:25–29.

Al-Majali, A. M., and S. Jawabreh. 2003. Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. *Small Rumin. Res.* **47**:243–248. doi:10.1016/S0921-4488(02)00259-6

Ariznabarreta, A., C. Gonzalo, and F. San Primitivo. 2002. Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci. *J. Dairy Sci.* **85**:1370–1375. doi:10.3168/jds.S0022-0302(02)74203-3

Arsenault, J., P. Dubreuil, R. Higgins, and D. Bélanger. 2008. Risk factors and impacts of clinical and subclinical mastitis in commercial meat-producing sheep flocks in Quebec, Canada. *Prev. Vet. Med.* **87**:373–393. doi:10.1016/j.prevetmed.2008.05.006

Barreiro, J. R., C. R. Ferreira, G. B. Sanvido, M. Kostrzewa, T. Maier, B. Wegemann, V. Böttcher, M. N. Eberlin, and M. V. dos Santos. 2010. Short Communication: Identification of subclinical cow mastitis pathogens in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Dairy Sci.* **93**:5661–5667. doi:10.3168/jds.2010-3614

Bhatt, V. D., V. B. Ahir, P. G. Koringa, S. J. Jakhesara, D. N. Rank, D. S. Nauriyal, A. P. Kunjadia, and C. G. Joshi. 2012. Milk microbiome signatures of subclinical mastitis-affected cattle analysed by shotgun sequencing. *J. Appl. Microbiol.* **112**:639–650. doi:10.1111/j.1365-2672.2012.05244.x

Blagitz, M. G., F. N. Souza, C. F. Batista, S. A. Diniz, J. P. Haddad, N. R. Benites, P. A. Melville, and A. M. Della Libera. 2014. Clinical findings related to intramammary infections in meat-producing ewes. *Trop. Anim. Health Prod.* **46**:127–132. doi:10.1007/s11250-013-0462-8

Bonsaglia, E. C. R., M. S. Gomes, I. F. Canisso, Z. Zhou, S. F. Lima, V. L. M. Rall, G. Oikonomou, R. C. Bicalho, and F. S. Lima. 2017. Milk microbiome and bacterial load following dry cow therapy without antibiotics in dairy cows with healthy mammary gland. *Sci. Rep.* **7**:8067. doi:10.1038/s41598-017-08790-5

Braem, G., S. De Vliegher, B. Verbist, V. Piessens, E. Van Coillie, L. De Vuyst, and F. Leroy. 2013. Unraveling the microbiota of teat apices of clinically healthy lactating dairy cows, with special emphasis on coagulase-negative staphylococci. *J. Dairy Sci.* **96**:1499–1510. doi:10.3168/jds.2012-5493

Casu, S., I. Pernazza, and A. Carta. 2006. Feasibility of a linear scoring method of udder morphology for the selection scheme of Sardinian sheep. *J. Dairy Sci.* **89**:2200–2209. doi:10.3168/jds.S0022-0302(06)72290-1

Clements, A. C., D. J. Taylor, and J. L. Fitzpatrick. 2003. Evaluation of diagnostic procedures for subclinical mastitis in meat-producing sheep. *J. Dairy Res.* **70**:139–148. doi:10.1017/s0022029903006022

Conington, J., G. Cao, A. Stott, and L. Bünger. 2008. Breeding for resistance to mastitis in United Kingdom sheep, a review and economic appraisal. *Vet. Rec.* **162**:369–376. doi:10.1136/vr.162.12.369

Cooper, S., S. J. Huntley, and L. E. Green. 2013. A longitudinal study of risk factors for teat lesions in 67 suckler ewes in a single flock in England. *Prev. Vet. Med.* **110**:232–241. doi:10.1016/j.prevetmed.2012.11.015

Crump, R. E., S. Cooper, E. M. Smith, C. Grant, and L. E. Green. 2019. Heritability of phenotypic udder traits to improve resilience to mastitis in Texel ewes. *Animal* **13**:1570–1575. doi:10.1017/S1751731118002951

Dorai-Raj, S. 2014. Binom: binomial confidence intervals for several parameterizations. Available from R package version 1.1-1. https://CRAN.R-project.org/package=binom. Accessed August 1, 2020.

Fernández, G., J. A. Baro, L. F. de la Fuente, and F. San Primitivo. 1997. Genetic parameters for linear udder traits of dairy ewes. *J. Dairy Sci.* **80**:601–605. doi:10.3168/jds.S0022-0302(97)75976-9

Fthenakis, G. C., E. T. el-Masannat, J. M. Booth, and J. E. Jones. 1991. Somatic cell counts of ewes' milk. *Br. Vet. J.* **147**:575–581. doi:10.1016/0007-1935(91)90029-M

Fthenakis, G. C., and J. E. Jones. 1990. The effect of experimentally induced subclinical mastitis on milk yield of ewes and on the growth of lambs. *Br. Vet. J.* **146**:43–49. doi:10.1016/0007-1935(90)90075-E

González-Rodríguez, M. C., C. Gonzalo, F. San Primitivo, and P. Cármenes. 1995. Relationship between somatic cell count and intramammary infection of the half udder in dairy ewes. *J. Dairy Sci.* **78**:2753–2759. doi:10.3168/jds.s0022-0302(95)76906-5

Gross, S. J., E. J. Pollak, J. G. Anderson, and D. T. Torell. 1978. Incidence and importance of subclinical mastitis in sheep. *J. Anim. Sci.* **46**:1–8. doi:10.2527/jas1978.4611

Hariharan, H., W. Donachie, C. Macaldowie, and G. Keefe. 2004. Bacteriology and somatic cell counts in milk samples from ewes on a Scottish farm. *Can. J. Vet. Res.* **68**:188–192.

Hueston, W. D. 1980. *A survey of subclinical mastitis in a ewe flock* [master's thesis]. Columbus (OH): The Ohio State University.

Huntley, S. J., S. Cooper, A. J. Bradley, and L. E. Green. 2012. A cohort study of the associations between udder conformation, milk somatic cell count, and lamb weight in suckler ewes. *J. Dairy Sci.* **95**:5001–5010. doi:10.3168/jds.2012-5369

Keisler, D. H., M. L. Andrews, and R. J. Moffatt. 1992. Subclinical mastitis in ewes and its effect on lamb performance. *J. Anim. Sci.* **70**:1677–1681. doi:10.2527/1992.7061677x

Kirk, J. H., E. M. Huffman, and B. C. Anderson. 1980. Mastitis and udder abnormalities as related to neonatal lamb mortality in shed-lambed range ewes. *J. Anim. Sci.* **50**:610–616. doi:10.2527/jas1980.504610x

Koop, G., J. F. Rietman, and M. C. Pieterse. 2010. *Staphylococcus aureus* mastitis in Texel sheep associated with suckling twins. *Vet. Rec.* **167**:868–869. doi:10.1136/vr.c3375

Lafi, S. Q., A. M. Al-Majali, M. D. Rousan, and J. M. Alawneh. 1998. Epidemiological studies of clinical and subclinical ovine mastitis in Awassi sheep in northern Jordan. *Prev. Vet. Med.* **33**:171–181. doi:10.1016/s0167-5877(97)00048-2

Legarra, A., and E. Ugarte. 2005. Genetic parameters of udder traits, somatic cell score, and milk yield in Latxa sheep. *J. Dairy Sci.* **88**:2238–2245. doi:10.3168/jds.S0022-0302(05)72899-X

Maisi, P., J. Junttila, and J. Seppänen. 1987. Detection of subclinical mastitis in ewes. *Br. Vet. J.* **143**:402–409. doi:10.1016/0007-1935(87)90017-0

McCarthy, F. D., J. B. Lindsey, M. T. Gore, and D. R. Notter. 1988. Incidence and control of subclinical mastitis in intensively managed ewes. *J. Anim. Sci.* **66**:2715–2721. doi:10.2527/jas1988.66112715x

McLaren, A., K. Kaseja, J. Yates, S. Mucha, N. R. Lambe, and J. Conington. 2018. New mastitis phenotypes suitable for genomic selection in meat sheep and their genetic relationships with udder conformation and lamb live weights. *Animal* **12**:2470–2479. doi:10.1017/S1751731118000393

Mørk, T., S. Waage, T. Tollersrud, B. Kvitle, and S. Sviland. 2007. Clinical mastitis in ewes; bacteriology, epidemiology and clinical features. *Acta Vet. Scand.* **49**:23. doi:10.1186/1751-0147-49-23

Persson, Y., A. K. Nyman, L. Söderquist, N. Tomic, and K. P. Waller. 2017. Intramammary infections and somatic cell counts in meat and pelt producing ewes with clinically healthy udders. *Small Rumin. Res.* **156**:66–72. doi:10.1016/j.smallrumres.2017.09.012

R Core Team. 2019. *R: a language and environment for statistical computing*. Vienna (Austria): R Foundation for Statistical Computing. Available from http://www.R-project.org/. Accessed August 1, 2020.

Riggio, V., L. L. Pesce, S. Morreale, and B. Portolano. 2013. Receiver-operating characteristic curves for somatic cell scores and California mastitis test in Valle del Belice dairy sheep. *Vet. J.* **196**:528–532. doi:10.1016/j.tvjl.2012.11.010

Spanu, C., Y. M. Berger, D. L. Thomas, and P. L. Ruegg. 2011. Impact of intramammary antimicrobial dry treatment and teat sanitation on somatic cell count and intramammary infection in dairy ewes. *Small Rumin. Res.* **97**:139–145. doi:10.1016/j.smallrumres.2011.03.005

Suarez, V. H., M. R. Busetti, A. O. Miranda, L. F. Calvinho, D. O. Bedotti, and V. R. Canavesio. 2002. Effect of infectious status and parity on somatic cell count and California mastitis test in pampinta dairy ewes. *J. Vet. Med. B. Infect. Dis. Vet. Public Health* **49**:230–234. doi:10.1046/j.1439-0450.2002.00552.x

Świderek, W. P., K. M. Charon, A. Winnicka, J. Gruszczyńska, and M. Pierzchała. 2016. Physiological threshold of somatic cell count in milk of Polish Heath Sheep and Polish Lowland Sheep. *Ann. Anim. Sci.* **16**:155–170. doi:10.1515/aoas-2015-0071

Torres-Hernandez, G., and W. Hohenboken. 1979. Genetic and environmental effects on milk production, milk composition and mastitis incidence in crossbred ewes. *J. Anim. Sci.* **49**:410–417. doi:10.2527/jas1979.492410x

USDA AMS. 2020. *National lamb market summary*.

USDA APHIS. 2012. Sheep 2011, part II: reference of marketing and death loss. USDA–APHIS–VS–CEAH–NAHMS. Fort Collins, CO. #632.1112.

Watkins, G. H., A. R. Burriel, and J. E. Jones. 1991. A field investigation of subclinical mastitis in sheep in southern England. *Br. Vet. J.* **147**:413–420. doi:10.1016/0007-1935(91)90083-Y

Watson, D. L., N. A. Franklin, H. I. Davies, P. Kettlewell, and A. J. Frost. 1990. Survey of intramammary infections in ewes on the New England Tableland of New South Wales. *Aust. Vet. J.* **67**:6–8. doi:10.1111/j.1751-0813.1990.tb07381.x

Werner, G., C. Fleige, A. T. Fessler, M. Timke, M. Kostrzewa, M. Zischka, T. Peters, H. Kaspar, and S. Schwarz. 2012. Improved identification including MALDI-TOF mass spectrometry analysis of group D streptococci from bovine mastitis and subsequent molecular characterization of corresponding *Enterococcus faecalis* and *Enterococcus faecium* isolates. *Vet. Microbiol.* **160**:162–169. doi:10.1016/j.vetmic.2012.05.019

Woodward, W. D., A. C. Ward, L. K. Fox, and L. B. Corbeil. 1988. Teat skin normal flora and colonization with mastitis pathogen inhibitors. *Vet. Microbiol.* **17**:357–365. doi:10.1016/0378-1135(88)90049-1