J. Dairy Sci. 109:690–704
https://doi.org/10.3168/jds.2025-27075

Subclinical mastitis in New Zealand grazing dairy ewes 1: Prevalence and risk factors

Greg Chambers,[1]* Kevin Lawrence,[2] Alex Grinberg,[2] Niluka Velathanthiri,[2] Anne Ridler,[2] and Richard Laven[2]
[1]EpiVets, Te Awamutu, New Zealand 3800
[2]School of Veterinary Science, Massey University, Palmerston North 4410, New Zealand

ABSTRACT

Our objectives were to describe subclinical mastitis and identify its risk factors among grazing dairy ewes in New Zealand. Gland-level milk samples were collected from ~15 randomly selected ewes on each of 20 dairy sheep farms at early, mid, and late lactation in a repeated cross-sectional study. California Mastitis Tests (CMT; measured on a scale of 0, trace, 1, 2, or 3) and aerobic bacterial culture were performed at the gland level, and SCC at the ewe level using composite milk samples. Subclinical mastitis was defined at the ewe level as having 1 or 2 bacteriologically positive glands and SCC >500 × 10^3 cells/mL or a CMT score ≥1 (or both). Milk samples were collected from 893 ewes, and complete subclinical mastitis data were available for 856 ewes. Median (range) SCC was 128,000 (2,000–34,953,000) cells/mL. A CMT score ≥1 in one or both glands was found in 21.2% of ewes. Bacteria were isolated from 5.5% of glands, with the most common species being non-*aureus* staphylococci (4.0% of glands) and *Staphylococcus aureus* (0.6% of glands). The prevalence of subclinical mastitis was 6.4% (95% CI = 4.6%–8.7%) and was not strongly clustered within farms (intraclass correlation coefficient = 0.04). Ewes with moderate or severe teat end hyperkeratosis had 6.4 times higher odds of subclinical mastitis than ewes with no or mild hyperkeratosis, and ewes with asymmetric udders had 2.3 times higher odds. The odds declined across the 3 visits. The prevalence was low compared with studies of more intensively farmed ewes in the northern hemisphere, but the bacterial causes were consistent. Subclinical mastitis management should be focused at the ewe level before the farm level, given the weak clustering within farms. When addressing or preventing a subclinical mastitis challenge, producers should consider teat end hyperkeratosis and udder asymmetry as simple visual screening tools but not rely on them alone to identify ewes at risk of subclinical mastitis. We present new information for New Zealand grazing dairy ewes, examine udder asymmetry as a diagnostic tool for subclinical mastitis, and show that, although prevalence was lower in New Zealand, the dominant pathogens are consistent, supporting the broader relevance of these findings to international mastitis control, albeit with adaptations for pasture-based systems.

Key words: sheep, milk quality, mastitis, somatic cell count

INTRODUCTION

Compared with the dairy cow industry, published research on milk quality and mastitis in dairy ewes is scarce, particularly for grazing systems. Commercial dairy sheep farms have emerged recently in New Zealand, where grazing management systems predominate. An estimated 30,000 ewes were being milked on ~30 farms in 2022 (McCoard et al., 2023), most of which were established after 2010. Modern facilities and equipment are therefore commonplace, and machine milking is standard. However, the gap in milk quality and mastitis data specific to grazing dairy sheep leaves producers and advisors reliant on extrapolation from bovine studies or from dairy sheep systems different to their own. Although literature specifically pertaining to grazing ewes exists from Mediterranean regions such as Sardinia (Cuccuru et al., 2011), Italy (Bianchi et al., 2004), Spain (Gonzalo et al., 2002), and Greece (Vasileiou et al., 2019a), the industries in these countries are longer-established than in New Zealand, and the extent of grazing, when stated, is often seasonal or only allowed during certain times of the day. In contrast, New Zealand's industry is young, almost entirely grazing-based, and typically uses modern infrastructure (McCoard et al., 2023). These differences in farm system, management, and industry stage mean that findings from Europe are not always transferable to New Zealand conditions.

Received June 10, 2025.
Accepted October 11, 2025.
*Corresponding author: greg@epivets.co.nz

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Subclinical mastitis is a significant concern for sheep dairy producers. Defined as intramammary infection without visible signs of inflammation in the udder or milk, it has been shown to affect milk quantity and quality (Leitner et al., 2004; Alba et al., 2019; Michael et al., 2023b), and the quality of products made from sheep milk such as cheese (Jaeggi et al., 2003). Research definitions of subclinical mastitis vary but require some combination of a positive milk culture, usually in the presence of elevated SCC (Fthenakis, 1994; Lysitsas et al., 2024), California Mastitis Test (**CMT**; Las Heras et al., 1999), or elevated milk neutrophil and lymphocyte proportions (Vasileiou et al., 2018).

In a study of 111 Greek dairy sheep flocks from a range of management systems, the prevalence of subclinical mastitis across all farms was 26%, and the most common pathogens were NAS, followed by *Staphylococcus aureus*, *Streptococcus* spp., and *Corynebacterium* spp. (Vasileiou et al., 2018). The same study found intensive management systems and hand milking to be risk factors for subclinical mastitis, and a subsequent analysis found that the risk was affected by ambient temperatures (Vasileiou et al., 2019a). Michael et al. (2023b) identified older age at which lambs are removed from ewes, anti-mastitis vaccination, and the employment of farm staff to be negatively associated with the risk of subclinical mastitis. Las Heras et al. (1999) found a lower risk when mechanical milking was used, as well as breed differences in the prevalence of subclinical mastitis. In low-input production systems, SCC was higher among primiparous ewes and when temperature–humidity indices were higher (Tzanidakis et al., 2021).

Our objectives were to systematically describe milk bacteriology results and the prevalence of, and risk factors for, subclinical mastitis among grazing dairy ewes on multiple New Zealand farms. This fills a global gap by focusing on pasture-based systems outside Europe.

MATERIALS AND METHODS

All animal manipulations in this study were approved by the Massey University Animal Ethics Committee (application AEC 22/25).

Sample Size

The number of commercial dairy sheep farms in New Zealand was unknown when this study was developed. In 2019, there were 18 known farms (Ministry for Primary Industries and Massey University, 2020), and, given the rapid expansion, ~40 commercial farms were estimated at the start of the 2022–2023 milking season. With a large proportion of farms being in the first or second season of production, a target of 20 farms (50% of New Zealand dairy sheep farms) was set.

The prevalence of subclinical mastitis was the primary outcome of this study. Because a 2-stage sampling method was employed (selection of ewes within farms), we used the R package epiR (Stevenson et al., 2024) to calculate the precision achieved with varying numbers of ewes per farm, based on the formula detailed by Bennett et al. (1991):

$$c = \frac{p\left(1-p\right)D}{s^2 b},$$

where c = the number of clusters (farms), p = the estimated prevalence, s = the standard error, b = the number of ewes sampled per farm, and D = the design effect, calculated from the formula

$$D = 1 + (b - 1)\,\rho,$$

where ρ is the intraclass correlation coefficient (**ICC**). Assuming a prevalence of 26% (Vasileiou et al., 2018) and an ICC of 0.06 (Barkema et al., 1997), a sample size of 30 ewes per farm per visit (1,800 ewes in total across 3 visits) would have allowed a 26% prevalence to be estimated with a 95% confidence interval that has a precision (half the width of the 95% CI) of 5.9%. Initially, we aimed to select 30 ewes per visit, but, after visiting 3 farms during visit 1, this was reduced to a minimum of 15 ewes because of time constraints and concerns about the length of time the ewes were held off pasture. Enrolling 15 ewes per visit from 20 farms was calculated to have a precision of 6.8%.

Study Design, Setting, and Participants

We conducted a repeated cross-sectional study on 20 commercial New Zealand sheep milking farms. The farms were selected to represent a range of locations and systems and have been previously described (Chambers et al., 2025). In brief, the farms were located in the North and South Islands between 37° and 44° south and 172° and 176° east (a north–south range of ~750 km). All farms were seasonal, and lambing occurred entirely in the spring except for 1 farm that also had an autumn-lambing flock. The median peak number of ewes milked per farm was 790 ewes, ranging from 171 to 1,530 ewes. All ewes lambed outdoors except on 3 farms, which lambed selected ewes indoors (e.g., ewes bearing triplets, 1-yr-old ewes, or other ewes during bad weather).

Visits were planned on 3 occasions on each farm during the 2022–2023 lactation season: August to October 2022 (visit 1), November to December 2022 (visit 2), and

March 2023 (visit 3), corresponding to the early, mid, and late lactation periods respectively. Visit 1 was scheduled to occur after the lambing period had ended but was skipped for farms that had not yet started to milk ewes due to a policy of rearing lambs on the ewes (n = 3 farms) or prolonged adverse weather (n = 1 farm).

On the first 3 farms, at the first visit, ewes were examined before the morning milking. Thereafter, examinations occurred 2 to 3 h after the morning milking to avoid prolonging milking time, except for 1 farm, where ewes were examined before morning milking at all 3 visits.

All ewes that lambed in the spring of the 2022–2023 season and were being milked at the time of each visit were eligible. Ewes were excluded if (1) they were under treatment or had been treated within the previous 30 d for illness; (2) they were diagnosed with clinical mastitis on the day of sampling (defined as visual or palpable udder changes with clots in the milk); (3) they were fractious and could not be safely examined or sampled; or (4) the ewe's teats, udder, or hindlegs were so heavily contaminated with moisture, dirt or feces that the operator deemed it unlikely that milk samples would be uncontaminated. However, no ewes presented with any of these exclusion criteria.

Study Procedures

On-Farm Procedures. Ewes were uniquely identified with existing visual ear tags. They were randomly selected using a calculation based on the total number of ewes being milked at the time of the visit, as described by Chambers et al. (2025). In brief, for the first 2 farms at visit 1, the number of ewes being milked was divided by 30 (the number of ewes to examine) to calculate the number p. The position of the first ewe to be selected was randomly chosen with a random number generator. Every pth ewe was selected in the parlor during milking. If a ewe was excluded, the adjacent ewe in the parlor was selected. On the third farm visited at visit 1, the same process was followed but the ewes were separated at the milking before the visit. For all other visits, on the day before the visit, the number of ewes being milked was divided by 18 to calculate p, and the same process was followed as above, providing 15 ewes and 3 spares. The first 15 eligible ewes were examined. The producers were responsible for selecting and separating ewes.

All procedures were carried out in the milking parlor by trained technicians or the lead author, with ewes in a standing position. Body fat reserves were assessed by assigning BCS on a 5-point scale, with increments of 0.5 (Kenyon et al., 2014). Teat and udder morphology and pathology assessments were performed as described by Chambers et al. (2025). Briefly, morphological assessments included teat length and width (mm) and udder depth, udder suspension, udder separation, and teat placement measured on a 5-point scale (Griffiths et al., 2019). Udder symmetry was subjectively assessed as either symmetrical or asymmetrical. Pathology assessments comprised presence of lesions of the teats and udder (non-mutually exclusively categorized as the presence or absence of nodules, scabs, scars, or other), teat and udder palpation findings, presence of teat and udder inflammation, and teat end hyperkeratosis. Glands were given palpation scores from 1 (soft consistency) to 7 (diffuse hard consistency), and teats were given palpation scores from 1 (soft consistency) to 5 (obstructed; Griffiths et al., 2019). Subsequently, udder palpation scores were collapsed into "normal" (1–2), "lump" (3–6), or "hard" (7), and teat palpation scores were collapsed into "normal" (1) or "abnormal" (2–5) using the method of Griffiths et al. (2019). In addition, thickening of the teat canal (present or absent) and teat canal patency (patent or blocked) were recorded. Hyperkeratosis was measured on a 4-point scale on farm and subsequently categorized into 3 ewe-level scores using the method of Vouraki et al. (2018): group 1, no or mild hyperkeratosis (both teat ends scored <3); group 2, medium hyperkeratosis (only 1 teat end scored ≥3); and group 3, severe hyperkeratosis (both teat ends scored ≥3).

Milk samples were collected from both glands, with each ewe's first teat being cleaned with alcohol-impregnated dry cow therapy teat wipes (Mediwipes, Mediray, Auckland, New Zealand) and sampled before moving to the second teat. For microbiological examination, duplicate samples of ~3 mL of milk were collected aseptically from each gland in 30-mL factory clean polycarbonate specimen vials (LabServ, Thermo Fisher Scientific, Auckland, New Zealand). A 25-mL sample was then collected separately from each gland into a measuring jug for CMT and SCC. Approximately 5 mL was used to perform the CMT while on farm. The CMT was measured on a 5-point scale (negative, trace, 1, 2, or 3) as described by Schalm and Noorlander (1957). The remaining milk from each gland was then combined into a single composite sample, gently mixed, and then divided into 2 polypropylene vials (37 mL each; Tekplas) for SCC analysis.

Immediately after collection, all samples were placed in a cooler box with ice and transported to the research center (EpiVets, Te Awamutu, New Zealand). Samples for SCC determination were transported to MilkTestNZ (Hamilton, New Zealand) in ice-packed containers on the same day as collection, arriving within 24 h of collection. Samples for aerobic culture were frozen at −20°C upon arrival at the research center and shipped periodically on ice to Massey University (Palmerston North, New Zealand).

Producer Interviews. When known, ewe demographic information (age, number of fetuses at pregnancy di-

agnosis, number of lambs born, lambing date, and first milking date) was collected from the farm owners or managers, either by email or at an in-person interview by the lead author after all ewes had been dried off (May–June 2023).

Subclinical mastitis risk factor data were collected at the same interview using a standardized questionnaire. This included questions on farm management practices, milking procedures, flock characteristics, and ewe health. The questions and response options used in the analysis are summarized in Table 1. The questionnaire and the selection criteria for the variables used in the risk factor analysis are presented in Supplemental Table S1 (see Notes). Milking frequency was categorized as twice daily all season, twice daily for part of the season and then once daily, or once daily all season. If a farm milked only once daily during the drying-off process at the end of lactation (~1 wk), it was deemed to have milked twice daily all season.

Laboratory Procedures. Somatic cell count was measured at the ewe level and aerobic bacterial culture at the gland level. The SCC was determined using a Combifoss 7 machine (Foss, Cambridge, New Zealand). Aerobic culture was performed as described by Chambers et al. (2024). Briefly, 10 µL from each thawed sample was cultured on 5% sheep blood agar plates (Fort Richard, Auckland, New Zealand), at 35°C to 37°C for 40 to 48 h. A minimum of 1 colony type with 3 or more colonies was necessary for the plate to progress to the next stage, except where a colony morphologically resembled *S. aureus*, when only 1 colony was required (Gonzalo et al., 2019). Plates with 3 or more colony types were defined as contaminated and not further analyzed. When all colony types had <3 colonies (and none resembled *S. aureus*), the sample was classified as "no growth," and no further action was taken. If a colony type had ≥3 colonies (or resembled *S. aureus*), 1 isolated colony was picked and subcultured onto a new agar plate and incubated as previously described to generate an isolate. The isolates were identified using MALDI-TOF (Microflex LT Biotyper, Bruker Daltonics). When MALDI-TOF identified a best and a second-best match, the best match was accepted. Where the original culture was defined as contaminated, or isolates were not identifiable by MALDI-TOF, the secondary sample was thawed and cultured using the same procedure. In such cases, the result from the secondary sample was used in the analysis. Isolation of *Mycoplasma* spp. was not attempted.

Table 1. Farm-level subclinical mastitis risk factor questions asked in farmer interviews in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand

Question
Are automatic cup removers used?
Are inline milk meters used?
Average milk production per ewe in the previous season (L)
Effective farm size (Ha)
Is an automated feeding system used?
Part of the season teat spray is used (all season, part season, not used)
Interval from lambing to lamb removal (d)
Milking flock size at peak
Milking frequency (twice daily; twice daily for part of the season then once daily; once daily)
Number of flock milk recording tests in the 2022–2023 season
Number of milking staff (early lactation)
Number of milking staff (mid–late lactation)
Number of seasons the farm has operated (including 2022–2023 season)
Part of the season teat spray is used
System pressure (kPa)
System pulsation frequency
Teat spray type (chlorhexidine, iodine)
Total number of staff (early lactation)
Total number of staff (mid–late lactation)
Type of flow line (high/low)
Use of gloves in milking shed (yes, no, personal choice, other)

Statistical Analysis

Results of aerobic culture, SCC, and demographic data were collated as spreadsheets (Microsoft Excel) and imported into RStudio using R 4.2.2 for analysis (R Core Team, 2023). Data were collated and merged in wide format by uniquely identifying each ewe and visit on each farm, and then examined for completeness, duplication, consistency, and spurious values.

The number of days in milk at each visit was calculated as the number of days between the recorded lambing date (when known) and the visit date. The number of days between lambing and first milking was calculated as the difference between the recorded lambing date and the recorded first milking date (when both were known). Age was categorized into hoggets (1 yr of age at lambing), 2-tooths (2 yr of age at lambing), and mixed-age (older than 2-tooths). Between-visit differences in lambing date, age at lambing, interval from lambing to first milking, BCS, and days since lambing at the visit were tested with the Kruskal–Wallis rank sum test, whereas Fisher's exact test was used for number of lambs born.

We collected data at the gland level (CMT score, aerobic culture results, and many of the morphology and pathology assessments), ewe level (ewe demographic information, SCC, and some morphology and pathology assessments), and farm level (farm-level risk factors). Culture results were collapsed to the ewe level for diagnosis of subclinical mastitis. Ewes were regarded as having a positive bacterial culture if at least 1 gland had an identified bacterial isolate and was not contaminated after culturing the second sample. All morphology and pathology assessments were collapsed to ewe-level scores by taking the highest (or most pathological) categorized score of the 2 teats or glands, except for teat length and width, for which the median was used. If inflammation

or lesions were diagnosed in at least 1 teat or gland, the ewe was deemed positive.

Descriptive statistics were calculated for SCC, and SCC was categorized as "normal" ($\leq 500 \times 10^3$ cells/mL), intermediate (between 500×10^3 and 1×10^6 cells/mL), and "high" ($>1 \times 10^6$ cells/mL) as per Fragkou et al. (2014). Confidence intervals for the proportion of ewes in each SCC category and CMT score were calculated using the Wilson method (Wilson, 1927). Bacterial culture results were descriptively reported at the gland level. If 2 colony types were identified for a gland, MALDI-TOF results were reported for both. To explore between-farm variation in bacteriology, the results were categorized into NAS, *S. aureus*, and "other" (all other isolates excluding unidentifiable samples), and the prevalence of each was calculated and plotted by farm.

Subclinical Mastitis Prevalence. Subclinical mastitis was defined at the ewe level as a bacteriologically positive milk sample (as defined previously) in 1 or both glands, alongside a CMT score ≥1 or SCC $>500 \times 10^3$ cells/mL (Fragkou et al., 2014). A bacteriologically positive milk sample with no increased CMT score (<1) or SCC $<500 \times 10^3$ cells/mL was defined as indicating "mammary carriage" (Vasileiou et al., 2018) and deemed not to have subclinical mastitis. The proportions of ewes in each combination of low (SCC $\leq 500 \times 10^3$ cells/mL) and intermediate or high SCC, low (CMT <1) and high CMT, and positive and negative culture are presented in Table 4 for descriptive purposes.

An overall estimate of the prevalence of subclinical mastitis, adjusted for clustering of subclinical mastitis risk within farm, was provided by constructing a generalized linear regression model with no fixed effects and a random intercept for farm. The binary outcome variable was the presence or absence of subclinical mastitis. Using the emmeans package (Lenth, 2024), the population-average predicted probability of subclinical mastitis and its 95% CI were calculated after adjusting for the bias introduced when converting from the logit to the probability scale, accounting for both model and random intercept standard deviations (Booth and Hobert, 1998). The ICC (proportion of variance at the farm level) was calculated using the latent variable approach (Wu et al., 2012). The model was checked for outlying farms by plotting farm random effects. Overdispersion was appraised by calculating the ratio of the model deviance to the degrees of freedom, and by using the DHARMa package (Hartig, 2022) to produce multiple simulated data sets, supply them to the model, plot the SD of the residuals from each model, and compare them to the SD from the fitted model to ensure the simulated residual SD clustered around the model residual SD.

Subclinical Mastitis Prevalence Across Visits and Farms. Farm- and visit-level prevalences were calculated directly from the raw data. Because of 0% prevalence on 1 farm, the confidence intervals were calculated using a Bayesian method with a Jeffreys prior of beta (0.5, 0.5) for farm prevalences, but the Wilson method for visit prevalences. Between-visit differences in the prevalence of subclinical mastitis were tested by constructing a mixed logistic regression model in the same way as the overall model, but with a fixed effect for visit, thus managing the correlation of ewes within farms. The significance of the association between visit and subclinical mastitis was tested with the likelihood ratio test of this model and the intercept-only overall model of subclinical mastitis. The same diagnostic checks were performed as described earlier. Between-farm differences were tested with Fisher's exact test.

Risk Factors for Subclinical Mastitis. Subclinical mastitis risk factor analysis was conducted on a complete-case basis because all exposure variables except number of lambs born and days in milk at the visit had <1% missing values. The missingness was caused by missing samples or records and deemed to be missing at random. The number of lambs born and days in milk at the visit had 54% and 46% missingness, respectively, which was too great to impute. The following variables were explored both as numeric variables and as categorized variables (categories in brackets): age (hogget, 2-tooth, or mixed-age), BCS (1–2 or 2.5–4), udder depth, separation, suspension and teat placement scores (1–2, 3, or 4–5), gland palpation (normal or abnormal), teat end hyperkeratosis (group 1 or groups 2–3), number of lambs born (1, 2, ≥3), interval between lambing and lamb removal (<7, ≥7 d), number of milk recording events (0, >0), pulsation frequency (<130, ≥130/min), plant vacuum (<38, ≥38 kPa), and number of seasons the farm has operated (2–3, ≥4).

The analysis was initiated by exploring associations between each pair of candidate variables to identify correlation and potential confounding. For pairs of continuous variables, the Pearson correlation coefficient was calculated. For pairs of continuous and ordinal categorical variables or nominal variables with only 2 levels (binary variables), the polyserial correlation was calculated. For pairs of ordinal categorical variables or nominal variables with only 2 levels, the polychoric correlation was calculated. For nominal categorical variables (farm and visit), associations with categorical variables were tested with the chi-squared test or, if expected counts in any cell were <5, Fisher's exact test, and their associations with continuous variables were tested with the Kruskal–Wallis rank sum test. Then the association between each variable and subclinical mastitis was tested with the chi-squared test or Fisher's exact test (when cell counts were <5) for categorical variables and the Kruskal–Wallis rank sum test for continuous variables. Univariable

logistic regression models of subclinical mastitis were constructed for all variables.

Risk factors were identified using mixed-effects logistic regression models with a random intercept for farm. Candidate variables were selected from the univariable logistic regression models based on having an association with subclinical mastitis with $P < 0.2$, but all non-candidate variables were offered to the final model one at a time to check for association and confounding. Two models were constructed, one with and one without udder symmetry as a candidate variable because udder symmetry is a consequence of subclinical mastitis rather than a risk factor but is easily used by producers as a diagnostic tool. Models were constructed in a backward stepwise manner, starting with all selected variables. Variables were removed one at a time and retained if their removal decreased the model fit (likelihood ratio test $P < 0.05$) or if they confounded other variables (their presence caused a >15% change in any other coefficients). The same diagnostic procedures were applied as for the model of subclinical mastitis prevalence, and the assumption of a linear relationship between continuous explanatory variables and the logit was checked graphically. The predictive accuracy of the 2 models was compared by calculating the area under the receiver-operating characteristic curve (**ROC AUC**) for each model and testing the difference with the method described by DeLong et al. (1988).

RESULTS

Enrollment and Data

Across the 3 visits, 893 observations were made on 882 unique ewes. Eleven ewes were examined at 2 visits by chance; however, 3 of these lacked ear tags and may, in fact, represent 6 different untagged ewes. No ewes were excluded at the selection stage. Visits 1 through 3 were conducted from August 24 to October 6, 2022; November 7 to December 22, 2022; and January 25 to March 16, 2023, respectively. Outside of the first 3 farm visits, more than 15 ewes were examined at 5 farm visits due to farmer selection errors and having enough time to enroll more ewes. Only 12 ewes were examined at 1 farm visit, due to farmer error in separating the ewes from the main flock. Complete demographic and examination data were available for 337 observations. Subclinical mastitis and its risk factors were not assessed for 37 ewes due to missing SCC, CMT, or microbiology data, leaving data from 856 ewes.

The numbers of ewes examined on each farm at each visit are summarized in Chambers et al. (2025), and the reasons for missing data are summarized in Supplemental Table S2 (see Notes).

Farm and Ewe Information

Because ewes were randomly selected anew at each visit, between-visit differences exist in the lambing spread of the selected ewes, with median lambing dates of August 7 (range = July 17–September 19), August 26 (range = July 9–October 16), and August 22 (range = July 2–October 15) for visits 1, 2, and 3, respectively (Kruskal–Wallis $P < 0.001$). Of the 409 ewes with data on the number of lambs born, 138 (34%), 211 (52%), 57 (14%), and 3 (0.7%) ewes had singles, twins, triplets, and quadruplets, respectively. These proportions did not differ between visits (Fisher's exact test $P = 0.233$). The distributions of age at lambing, DIM at first milking, and BCS and DIM at the visit differed between visits (Table 2).

SCC

Across all visits, SCC data were available for 890 ewes, with a median (interquartile range) SCC of 128,000 (75,250–264,500), an arithmetic mean of 848,829, and a range of 2,000 to 34,953,000 cells/mL. There were 748, 53, and 89 samples with normal ($<500 \times 10^3$ cells/mL), intermediate (between 500×10^3 and 1×10^6 cells/mL), and high ($>1 \times 10^6$ cells/mL) SCC, respectively, corresponding to 84% (95% CI 81.5%–86.3%), 6% (95% CI 4.6%–7.7%), and 10.0% (95% CI 8.2%–12.1%) of samples, respectively.

Table 2. Median and range (in parentheses) of age, body condition score, days since lambing at visit, and days since lambing at the first milking of the season, overall and at each visit, in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand

Variable	N[1]	Overall	Visit 1	Visit 2	Visit 3	*P*-value[2]
Age at lambing (yr)	659	2 (1, 7)	3 (1, 7)	2 (1, 7)	2 (1, 7)	0.010
Interval from lambing to first milking (d)	438	3 (0, 161)	3 (1, 41)	4 (1, 126)	4 (0, 161)	<0.001
Body condition score at visit	890	3 (1, 4)	3 (2, 4)	3 (1, 4)	3 (2, 4)	<0.001
Days since lambing at visit	479	102 (8, 243)	32 (8, 64)	94 (28, 143)	183 (107, 243)	<0.001

[1]Numbers differ due to missing values (body condition score) or because not all farms collected these data (all other variables).

[2]Kruskal–Wallis rank sum test.

California Mastitis Test

Data were available for 1,757 glands from 885 ewes. There were 1,069 (60.8%, 95% CI 58.5%–63.1%), 418 (23.8%, 95% CI 21.9%–25.8%), 121 (6.9%, 95% CI 5.8%–8.2%), 86 (4.9%, 95% CI 4%–6%), and 63 (3.6%, 95% CI 2.8%–4.6%) glands having scores of 0, trace, 1, 2, and 3, respectively. At the ewe level, a CMT score ≥1 was detected in at least 1 gland of 189/893 (21.2%) ewes, with 108/189 (57.1%) being positive in a single gland (i.e., the other gland had a score <1).

Aerobic Culture Results

Milk samples from 1,763 glands from 884 ewes were submitted for aerobic culture; MALDI-TOF was performed on 103 samples after removing samples with no growth (n = 1,650) and contaminated first and second samples (n = 10). Bacteria were identified by MALDI-TOF in 97/1,763 (5.5%) glands. The MALDI-TOF analysis did not identify the isolates in 6 cases despite using the secondary sample. No further attempts were made to identify these isolates.

Non-*aureus* staphylococci were the most common isolates, being confirmed in 71/1,763 (4%) glands, followed by *S. aureus* in 10/1,763 (0.6%) glands. Other species (*Bacillus licheniformis*, *Citrobacter gillenii*, *Enterococcus hirae*, *Kocuria atrinae*, *Lactococcus lactis*, *Serratia marcescens*, *Serratia nematodiphila*, *Streptococcus infantarius*, *Streptococcus ovis*, and *Streptococcus uberis*) were found in 16/1,763 (0.9%) glands (summarized in Table 3). *Staphylococcus aureus* was found on 5/20 (25%) and NAS on 16/20 (75%) farms. The proportions of milk samples that were confirmed as NAS, *S. aureus*, and other are shown at the farm level in Figure 1.

Table 3. Results of microbiological culture of gland-level milk samples (n = 1,763) in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand

Bacteriology	N (%)
No growth	1,650 (94)
Staphylococcus warneri	17 (1.0)
Staphylococcus caprae	16 (0.9)
Staphylococcus aureus	10 (0.6)
Contaminated	10 (0.6)
Staphylococcus auricularis	7 (0.4)
Staphylococcus haemolyticus	7 (0.4)
Staphylococcus devriesei	6 (0.3)
No identification possible	6 (0.3)
Staphylococcus epidermidis	5 (0.3)
Staphylococcus simulans	5 (0.3)
Staphylococcus chromogenes	4 (0.2)
Streptococcus uberis	4 (0.2)
Escherichia coli	2 (0.1)
Staphylococcus xylosus	2 (0.1)
Streptococcus infantarius	2 (0.1)
Bacillus licheniformis	1 (<0.1)
Citrobacter gillenii	1 (<0.1)
Enterococcus hirae	1 (<0.1)
Kocuria atrinae	1 (<0.1)
Lactococcus lactis	1 (<0.1)
Serratia marcescens	1 (<0.1)
Serratia nematodiphila	1 (<0.1)
Staphylococcus caprae and *Staphylococcus warneri*	1 (<0.1)
Staphylococcus warneri and *Staphylococcus epidermidis*	1 (<0.1)
Streptococcus ovis	1 (<0.1)

Subclinical Mastitis

The numbers of ewes diagnosed with each combination of bacterial culture, dichotomized SCC, and dichotomized CMT results are shown in Table 4. Among the 89.8% of ewes that were culture-negative, 88.8% had SCC ≤500 × 10^3 cells/mL, and 81.1% had both SCC ≤500 × 10^3 cells/mL and a CMT score <1. Conversely, among the 10.2% of ewes that were culture-positive, only 59.8% had SCC >500 × 10^3 cells/mL, and only 56.3% had both SCC >500 × 10^3 cells/mL and CMT score ≥1.

We diagnosed subclinical mastitis in 58/856 (6.8%) ewes, and mammary carriage (a bacteriologically positive milk sample with CMT <1 and SCC <500 × 103 cells/mL) in 29/856 (3.4%) ewes. Thus 58/87 (67%) of bacteriologically positive ewes were diagnosed with subclinical mastitis. The overall subclinical mastitis prevalence computed by the mixed model, adjusting for clustering within farms, was 6.4% (95% CI = 4.7%–8.8%); the ICC was 0.04.

Subclinical mastitis prevalence declined numerically from visit 1 to visit 3, with prevalences of 9.0% (95% CI 6.1%–13.1%), 6.9% (95% CI 4.5%–10.3%), and 4.6% (95% CI 2.7%–7.7%) at visits 1 through 3, respectively, but a statistical difference was not confirmed (P = 0.125). At the farm level (Figure 2), prevalence ranged from 0.0% (95% CI = 0.0%–4.2%) to 16.7% (95% CI = 7.0%–28.8%), but no statistical difference between farms was confirmed (P = 0.126).

Risk Factors for Subclinical Mastitis

Descriptive information on ewe-level variables (age, BCS, days in milk, number of lambs born, and teat and udder morphology and pathology scores) can be found in Chambers et al. (2025). The distributions of risk factors that had unadjusted associations with subclinical mastitis with P < 0.2, and their distributions for ewes with and without subclinical mastitis, are shown in Tables 5 and 6. Distributions for all risk factors and univariable logistic regression models are included in Supplemental Tables S3, S4, and S5 (see Notes).

Positive subclinical mastitis associations (odds ratio [**OR**] >1) with P < 0.2 were identified for udder asymmetry, teat end hyperkeratosis (both in its original scale and dichotomized into group 1 and groups 2 and 3), milking

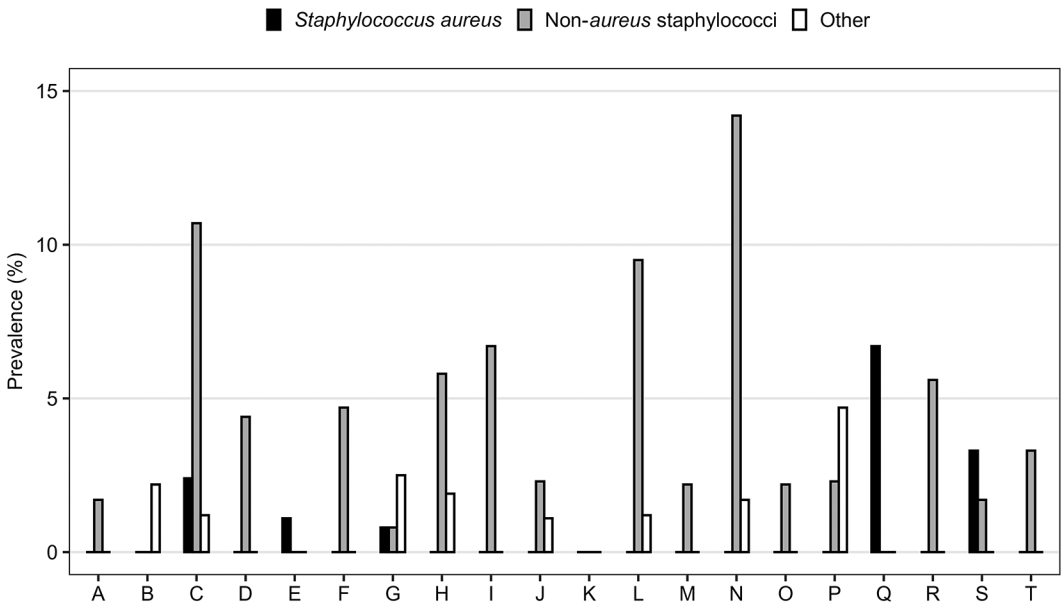

Figure 1. Percentage of milk samples on each farm that were confirmed as non-*aureus* staphylococci, *Staphylococcus aureus*, or other species (Other), in a study of udder health of randomly selected ewes (n = 873) on 20 commercial dairy sheep farms in New Zealand (A–T).

twice daily all season (compared with twice daily for part of the season then once daily, or once daily all season), system pressure, low udder separation scores, and a low number of seasons the farm has operated (2–3 seasons compared with 4–25). Negative associations (OR <1), with $P < 0.2$, were found for visit number, number of milking staff in mid–late lactation, low line milking systems (compared with high line), and teat spray use for the whole season (compared with part of the season or not used). The prevalence also varied between farms, with $P < 0.2$.

Number of milking staff in mid–late lactation and type of flow line (high vs. low) caused singularity issues so they were excluded from the models, and type of flow line was substituted with categorized system pressure.

The final statistical models, with and without udder symmetry included as a covariate (due to its being a consequence of mastitis rather than a risk factor), are summarized in Table 7. Teat end hyperkeratosis, categorized into group 1 (none or mild) and groups 2 and 3 (medium or severe), was the only variable included in the final model without udder symmetry. Ewes with group 2 or 3 hyperkeratosis had 6.4-fold (95% CI = 1.5–27.5) higher odds of subclinical mastitis compared with ewes with group 1 hyperkeratosis. When udder symmetry was included, teat end hyperkeratosis and visit were the only other variables in the final model. Ewes with group 2 or 3 hyperkeratosis had a 7.6-fold (95% CI = 1.7–34.6) increase in the odds of subclinical mastitis, and ewes diagnosed with asymmetric udders had 2.3-times (95% CI = 1.3–4.0) higher odds. The odds of subclinical mastitis more than halved across visits, with ewes at visit 3 having 0.4 times (95% CI = 0.2–0.8) the odds of subclinical mastitis than ewes at visit 1. The model with udder sym-

Table 4. Bacterial milk culture, SCC, and California Mastitis Test (CMT) results for ewes with complete culture, SCC, and CMT data (n = 856), in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand

		CMT,[2] n/n (%)	
Bacterial culture result, n/n (%)	SCC,[1] n/n (%)	Negative	Positive
Negative culture, 769/856 (89.8)	Low, 683/769 (88.8)	624/683 (91.4)	59/683 (8.6)
	High, 86/769 (11.2)	17/86 (19.8)	69/86 (80.2)
Positive culture, 87/856 (10.2)	Low, 35/87 (40.2)	29/35 (82.9)	6/35 (17.1)
	High, 52/87 (59.8)	3/52 (5.8)	49/52 (94.2)

[1]Dichotomized into low (≤500,000 cells/mL) or high (>500,000 cells/mL).
[2]Dichotomized into negative (0 or trace) or positive (1–3).

Table 5. Mean (range) of continuous variables that had univariable associations with subclinical mastitis ($P < 0.2$), overall and by ewe subclinical mastitis status, in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand[1]

Variable	N	Overall (n = 856)	Negative (n = 798)	Positive (n = 58)	*P*-value[2]
System pressure (kPa)	812	38.4 (32–43)	38.4 (32–43)	39.1 (32–43)	0.055
Total number of staff, mid–late lactation	856	3.5 (1–10)	3.5 (1–10)	3.3 (1–10)	0.12
Number of milking staff, mid–late lactation	856	2.2 (1–4)	2.2 (1–4)	2.1 (1–4)	0.11

[1]Numbers of observations differ due to missing data for some variables.
[2]Wilcoxon rank sum test.

metry had a ROC AUC of 0.71 (95% CI = 0.64–0.77), and the model without udder symmetry had a ROC AUC of 0.7 (95% CI = 0.63–0.76).

DISCUSSION

Our goal was to establish a baseline data set from grazing dairy ewes to better understand the extent and variability of subclinical mastitis within and between flocks, facilitate comparisons across farms, identify risk factors, pinpoint areas for improvement, support the planning of future studies, and enable progress assessment over time. Our study population comprised grazing New Zealand dairy ewes that were mostly or entirely grazing all year on farms that had been operating <10 years, complementing the bulk of the literature from more intensive, northern hemisphere flocks.

The prevalence of subclinical mastitis was 6.4%, varying from 4.3% to 8.4% between visits and from 0% to 17% between farms. This was low compared with European studies, although methodological and demographic differences may explain this. A large-scale study of Greek flocks estimated the prevalence at a single time point to be 26% (Vasileiou et al., 2018), varying relatively widely from 0% to 85% between farms. Earlier work in Greece found subclinical mastitis prevalences of 5%, 11%, and 17% in early, mid, and late lactation, respectively (Fthenakis, 1994), which are more consistent with the results of the present study. In contrast, Las Heras et al. (1999) estimated the prevalence to be 34% among Spanish ewes. The prevalence in the present study is low in comparison.

Some of the prevalence differences may be methodological due to varying techniques and definitions. Vasileiou et al. (2018) defined subclinical mastitis as a bacteriologically positive milk sample (>10 colonies of the same organism, instead of ≥3 in the present study) with no more than 2 colony types (consistent across both studies) and having a CMT score ≥1 (instead of a CMT score ≥1 or SCC >500 × 10^3 cells/mL, or both, in the present study) as well as altered neutrophil and lym-

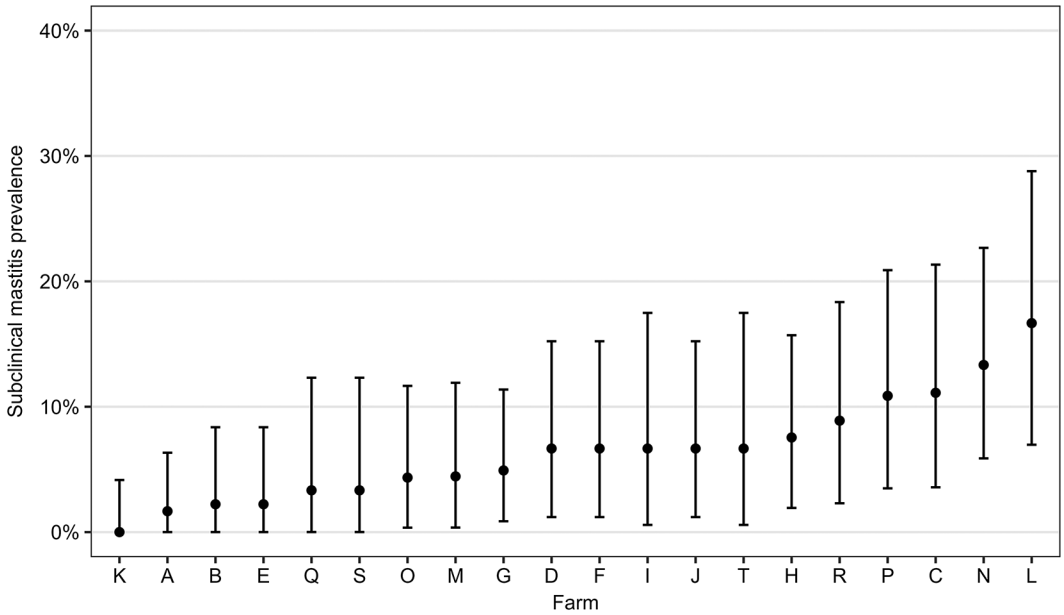

Figure 2. Prevalence of subclinical mastitis on each farm in a study of udder health of randomly selected ewes (n = 871) on 20 commercial dairy sheep farms in New Zealand (A–T). Error bars represent 95% CI.

Table 6. Distributions of categorical variables that had univariable associations with subclinical mastitis ($P < 0.2$), overall and by ewe subclinical mastitis status, in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand[1]

Variable	N	Overall (n = 856)	Negative (n = 798), n (%)	Positive (n = 58), n (%)	*P*-value
Visit	856				0.12[2]
1		266	242 (91)	24 (9.0)	
2		306	285 (93)	21 (6.9)	
3		284	271 (95)	13 (4.6)	
Udder symmetry	854				0.002[2]
Yes		528	504 (95)	24 (4.5)	
No		326	293 (90)	33 (10)	
Teat end hyperkeratosis[3]	846				0.028[4]
Group 1		837	782 (93)	55 (6.6)	
Group 2		6	4 (67)	2 (33)	
Group 3		3	2 (67)	1 (33)	
Teat spray use	856				0.13[2]
All season		358	341 (95)	17 (4.7)	
Part season		424	389 (92)	35 (8.3)	
Not used		74	68 (92)	6 (8.1)	
Milking frequency	856				0.13[2]
Twice daily		272	248 (91)	24 (8.8)	
Twice daily for part of the season then once daily		406	379 (93)	27 (6.7)	
Once daily		178	171 (96)	7 (3.9)	
Type of flow line (high/low)	856				0.014[2]
High		458	418 (91)	40 (8.7)	
Low		398	380 (95)	18 (4.5)	
Teat end hyperkeratosis (categorized)[1]	846				0.019[4]
Group 1		837	782 (93)	55 (6.6)	
Group 2 or 3		9	6 (67)	3 (33)	
No. seasons the farm has operated (including 2022–2023 season; categorized)	856				0.2[2]
2–3		476	439 (92)	37 (7.8)	
4–25		380	359 (94)	21 (5.5)	

[1]Numbers of observations differ due to missing data for some variables.

[2]Pearson's chi-squared test.

[3]Scored at the gland level on a scale of 1–4 (Vouraki et al., 2018); then classified at the ewe level into group 1 = no or mild hyperkeratosis (both teat ends <3), group 2 = medium hyperkeratosis (one teat end ≥3), or group 3 = severe hyperkeratosis (both teat ends ≥3).

[4]Fisher's exact test.

Table 7. Final mixed logistic regression models of the odds of subclinical mastitis, in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand[1]

	Model without udder symmetry		Model with udder symmetry	
Variable	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Teat end hyperkeratosis[2]				
Group 1	—		—	
Group 2 or 3	6.35 (1.47–27.5)	0.013	7.61 (1.68–34.6)	0.009
Visit				
1			—	
2			0.68 (0.36–1.27)	0.2
3			0.40 (0.19–0.84)	0.016
Udder symmetry				
Yes			—	
No			2.32 (1.33–4.04)	0.003

[1]OR = odds ratio.

[2]Scored at the gland level on a scale of 1–4 (Vouraki et al., 2018); then classified at the ewe level into group 1 = no or mild hyperkeratosis (both teat ends <3), group 2 = medium hyperkeratosis (one teat end ≥3), or group 3 = severe hyperkeratosis (both teat ends ≥3).

phocyte proportions. The present study therefore had a lower threshold for diagnosing subclinical mastitis, as it required a lower bacterial count and used SCC data but did not require altered leukocyte profiles.

Furthermore, we froze the milk samples before culture, which may have altered bacterial viability. The limited published research on the effect of freezing on culture of ewe milk suggests that freezing reduces the viability of some bacterial species in milk, although this may be more pronounced in low colony-forming unit count samples (Smith et al., 2011), and deterioration appears to be greater for gram-negative pathogens (Sánchez et al., 2003).

We did not sample all ewes on each farm, so random error may have contributed to the low prevalence. Although the 95% CI had a width of only 4% in absolute terms, expanding on this study by sampling all (or a higher proportion of) the ewes on several farms would provide greater confidence that the prevalence is indeed as low as 6.4%.

Farm management may also affect prevalence. All ewes in the present study were machine-milked on extensive farms. The New Zealand setting is unique due to the year-round grazing typically practiced on farms that are relatively young and therefore have modern facilities. Vasileiou et al. (2018) found a lower prevalence of staphylococcal subclinical mastitis among flocks that were machine-milked (but no difference in the prevalence of subclinical mastitis of any cause), and a lower prevalence of subclinical mastitis (overall and staphylococcal) among extensively managed flocks. Las Heras et al. (1999) also noted that the overall prevalence was lower among machine-milked ewes. None of the producers administered antimicrobial therapy aimed at subclinical mastitis at the end of lactation or mastitis vaccines, and New Zealand has no registered dry ewe therapies or mastitis vaccines for sheep, removing these 2 factors as potential confounders. This study supports the notion that the prevalence of subclinical mastitis is lower in grazing, machine-milked dairy sheep, but we did not include ewes that were intensively farmed or hand-milked, so we cannot confirm this.

Ewe demographics may also have contributed to the lower prevalence. The ewes in our study had a median age of 2 yr, possibly because more than half of the ewes were on farms in the first 2 to 3 seasons of production. The ages of ewes in other published studies have not always been reported. In the study of Fthenakis (1994), more than 50% of the ewes were in their fourth or greater parity, and the prevalence of subclinical mastitis was 0% to 11.4% among first- and second-parity ewes across the 3 visits, but 5.5% to 22.2% for third parity or greater. The lower prevalence in the present study may reflect the younger age of the ewes, but age was not included in the final model, although age data were only available for ~75% of ewes.

A between-farm difference was not confirmed. With an ICC of 0.04, little clustering of subclinical mastitis within farm occurred, meaning most of the variation was within farms rather than between farms (the prevalence of subclinical mastitis did not appear to have a strong association with unmeasured farm-level factors among the study farms). Clinical mastitis also had a low ICC of 0.054 among ewes on the same farms in the same season (Chambers et al., 2024). This suggests that, although farm-level practices remain important (e.g., use of vaccination or teat disinfection), prevention of subclinical mastitis should prioritize identifying and managing ewe-level risk factors and variation within farms (e.g., monitoring SCC).

Positive aerobic culture results (excluding contaminated samples and unidentifiable isolates) occurred in only 5.5% of glands and were dominated by NAS and *S. aureus*. A total of 10 *S. aureus* were isolated on 5 farms, suggestive of clustering, but, with a low prevalence of *S. aureus* IMI, more work is required to confirm clustering. *Streptococcus uberis* was one of the least frequently isolated species, in contrast to a study of clinical mastitis on the same farms in the same season, where it was the most common isolate (Chambers et al., 2024). The species composition was consistent with that seen by Greek researchers who also randomly sampled ewes (Fthenakis, 1994; Vasileiou et al., 2018; Michael et al., 2023a). However, researchers from the United States found that *Bacillus* spp. dominated the bacteriology (Knuth et al., 2019). It should be noted that we collected milk samples from randomly selected ewes and did not target ewes with risk factors for subclinical mastitis such as elevated SCC. Targeted selection would likely yield different bacteriological results.

The correlation between SCC and CMT, and optimal SCC thresholds for diagnosing intramammary infection, are presented in Chambers et al. (2026). In the present study, SCC and CMT were dichotomized, and the agreement between bacterial culture result, SCC, and CMT was greater among ewes with negative culture results than among those with positive results. Of the ewes with positive culture results, 52/87 (59.8%) had a high SCC, and 55/87 (63.2%) had a high CMT (score 1–3). Furthermore, 86/138 (62.3%) high-SCC ewes were culture-negative. These findings signal the limitations of a single culture, SCC measurement, or CMT score for accurately diagnosing subclinical mastitis. Intermittent shedding of bacteria, nonpathogenic infections, loss of bacterial viability, and delays in the return of SCC and CMT to normal values after infection resolution may explain these discrepancies.

Screening of 41 ewe- and farm-level variables as well as the categorized versions of 13 of those variables

found only 11 variables to have a univariable association with subclinical mastitis ($P < 0.2$), only 3 of which were confirmed as risk factors for subclinical mastitis (i.e., teat end hyperkeratosis, udder asymmetry, and visit were retained in the final model). When udder symmetry was included in the model, visit, teat end hyperkeratosis, and udder asymmetry were all identified as risk factors, whereas without udder symmetry, teat end hyperkeratosis was the only identified risk factor. Michael et al. (2023b) screened 67 variables and found only 3 to have an association (younger age of newborns when taken away from the dam, omission of anti-staphylococcal mastitis vaccination of ewes, and lack of employed staff on the farms). The lack of other risk factors identified in the present study may be due to a true lack of association or to the low prevalence of subclinical mastitis and insufficient study power.

Vouraki et al. (2018) identified teat end hyperkeratosis as a risk factor for subclinical mastitis (defined as an elevated CMT alone). Their reported effect size (OR of 1.4 and 1.6 for medium and severe hyperkeratosis, respectively) was lower than the 7.6 we found. However, the prevalence of medium or severe hyperkeratosis in our study was very low, 1.1% compared with the 17.4% reported by Vouraki et al. (2018), which means that our estimate of the effect of teat end hyperkeratosis on the risk of subclinical mastitis has a wide CI (1.7–34.6). Furthermore, our definition of subclinical mastitis (culture-positive with elevated CMT or SCC) was not the same as the CMT alone used by Vouraki et al. (2018), with only 55 of the 183 ewes (29.8%) that we identified with an increased CMT score (score 1–3) actually being culture-positive.

To our knowledge, no similar data have been published on the association between udder asymmetry and subclinical mastitis in dairy ewes. Margatho et al. (2020) showed that udder asymmetry in Serrana goats was associated with a higher SCC. However, we cannot directly compare the results due to the different methodology.

Including udder symmetry (and visit) in the model with teat end hyperkeratosis did not improve the ROC AUC for detecting subclinical mastitis (0.71 vs. 0.70, respectively). Only 3/58 (5.2%) ewes diagnosed with subclinical mastitis had group 2 or 3 hyperkeratosis, so screening for subclinical mastitis by detecting hyperkeratosis has a very low sensitivity. Our data suggest that, although farmers need to recognize teat end hyperkeratosis (and understand its importance), when prevalence is low, screening for teat end hyperkeratosis is not likely to be a useful test for subclinical mastitis. In contrast 33/57 (58%) ewes with subclinical mastitis had udder asymmetry, meaning it has a higher sensitivity. Given that the prevalence of asymmetry was 39% in the present study, screening ewes for asymmetry may be a useful tool for identifying ewes at risk of subclinical mastitis, trading accuracy for ease. However, only 33/326 (10%) ewes with asymmetric udders had subclinical mastitis, meaning it has a very low positive predictive value. These animals should therefore receive a more specific test to confirm the diagnosis of subclinical mastitis.

Our visits took place at early, mid, and late lactation, in line with the subclinical mastitis work of Fthenakis (1994) in the Greek milking sheep flock, which found an increase in the prevalence of subclinical mastitis over time as lactation progressed. Defined as milk of normal appearance that was bacteriologically positive and had SCC $>1 \times 10^6$ cells/mL, the prevalence of subclinical mastitis increased from 4.5% to 16.9% across 3 visits performed over 8 to 11 weeks. This pattern was also reported by Mavrogianni et al. (2007), who showed a declining hazard of teat ducts and mammary secretions remaining uninfected across lactation. In contrast, the prevalence declined over time in our data set, with visit remaining in the final prevalence model when udder symmetry was included as a factor. It is unclear why there was a difference in the effect of lactation stage on the risk of subclinical mastitis in dairy ewes between our study and those of Fthenakis (1994) and Mavrogianni et al. (2007).

One potential difference is that, in contrast to Fthenakis (1994), who sampled the same ewes across their study, we randomly selected animals anew at each visit. This introduced the possibility of chance playing a role or ewes with subclinical mastitis being removed from the sample population. It also means that between-visit demographic differences were observed. In particular, median age was older among ewes sampled at the first visit because hoggets (1-year-olds) typically lamb ~1 mo later than older ewes, and therefore many were not present in the milking flock at the first visit. As increased age appears to be a risk factor for udder infection (Vasileiou et al., 2019b), this reduction in average age over time may explain our finding that, contrary to Fthenakis (1994), we did not find an increase in subclinical mastitis prevalence as lactation progressed. Furthermore, although we did not find an association ($P < 0.2$) between age and prevalence of subclinical mastitis at the univariable level (Supplemental Table S5), the lack of such an association does not rule out a potentially large association. For example, compared with hoggets, at the univariable level, the odds of subclinical mastitis in 2-tooth ewes was 2.08 times higher, with the 95% CI of this estimate being 0.84 to 5.62. Thus, our data (at the univariable level) were compatible with a large increase in the odds of subclinical mastitis with age. We need further data on the association between lactation stage and risk of subclinical mastitis in grazing dairy ewes.

Producers seeking to reduce the prevalence of subclinical mastitis in their flocks need to accurately identify affected ewes. We defined subclinical mastitis as a positive bacterial culture in the presence of elevated SCC or CMT, or both, and identified udder asymmetry and teat end hyperkeratosis as potent risk factors for subclinical mastitis. However, these 2 factors alone should not be used to make management decisions (such as treatment, drying off, or culling), because their presence does not guarantee that a ewe has subclinical mastitis, nor does their absence guarantee that a ewe does not have subclinical mastitis. We recommend they be used as screening tools in conjunction with SCC and milk culture. When using SCC data alone, it is not clear what threshold to apply or how many SCC measurements are required to be confident that a ewe does or does not have subclinical mastitis. Chambers et al. (2026) proposed an SCC threshold based on a single SCC measurement from the same data set. Berthelot et al. (2006), however, proposed that at least 2 SCC measurements are required to diagnose the subclinical mastitis status of dairy ewes. This seems prudent if a producer is deciding whether to dry off, treat, or cull a ewe.

Our conclusions are limited by the low prevalence of subclinical mastitis and the completeness of farm records. More than half of the farms were in the first 2 to 3 seasons of production, which meant that some data were missing because of incomplete farm data recording and collection systems. This was especially true for ewe demographic data because many farmers did not record it at the individual ewe level. In contrast, only small proportions of milk data were missing (up to 19/893 observations, 2.1%), which we believe had minimal influence on the conclusions drawn from this study.

Visits were made 2 to 3 h after milking on all farms except one, where they were made before milking. The different timing of examination and sample collection on this farm may have affected the variables we measured, but it is not possible to quantify this effect because of confounding by other unmeasured factors on the farm we visited before milking. We intended to sample a larger number of ewes (n = 30) per farm, but we reduced the sample size per farm due to the long amount of time sampling took and the attendant animal welfare risks. This reduction in sample size did not substantially affect the estimated precision of our subclinical mastitis prevalence.

We have found both similarities and differences between New Zealand grazing systems and established European and US systems. Although this study focused on subclinical mastitis among New Zealand grazing dairy ewes that typically spent most, if not all, of their time on pasture on farms that had been in operation for <10 years, the data provide useful information for other grazing systems globally. Our sample size was limited by the smaller scale of the industry in New Zealand, yet we enrolled approximately half of the commercial farms operating at the time. The prevalence of subclinical mastitis was lower than that reported elsewhere, but the bacteriology was similarly dominated by NAS and *S. aureus*. Teat end hyperkeratosis was confirmed in our study to be a risk factor for subclinical mastitis, consistent with previous work, and udder asymmetry has not been studied elsewhere to our knowledge. This suggests that, although pathogen-directed mastitis control principles developed in Mediterranean systems remain relevant globally, their implementation may need to be adapted for pasture-based, machine-milked flocks such as those in New Zealand.

CONCLUSIONS

The prevalence of subclinical mastitis was low in grazing New Zealand dairy ewes compared to overseas research in non-grazing ewes. Bacteriology was dominated by NAS and *S. aureus*, indicating that the bacterial causes of subclinical mastitis in pasture-based ewes are not substantially different from those in more intensive systems. Teat end hyperkeratosis was a potent but rare risk factor for subclinical mastitis, whereas ewes with asymmetric udders had substantially higher odds of subclinical mastitis than ewes with symmetric udders. The diagnostic values of teat end hyperkeratosis and udder symmetry were at best moderate, underscoring the importance of measuring SCC. These findings provide a benchmark for udder health in grazing dairy ewes. The similarity in dominant pathogens to those reported in established Mediterranean systems underscores the wider relevance of this work. However, differences in prevalence and ewe-level risk factors highlight the need to adapt international mastitis control strategies to the realities of pasture-based, machine-milked flocks.

NOTES

Funding for this study was provided by AGMARDT (Feilding, New Zealand), Boehringer Ingelheim (Auckland, New Zealand), EpiVets (Te Awamutu, New Zealand), Massey University (Palmerston North, New Zealand), Maui Sheep Milk (Hamilton, New Zealand), MilkTestNZ (Hamilton, New Zealand), the New Zealand Veterinary Association (Wellington, New Zealand), Sheep Milk New Zealand (Christchurch, New Zealand), Spring Sheep (Hamilton, New Zealand), and Virbac (Hamilton, New Zealand). The authors acknowledge the help provided by Sarah Hurst (EpiVets, Te Awamutu, New Zealand) for leading the on-farm technician work, and Chloe Ashworth (VetEnt, Ashburton, New Zealand),

Marion Benoit (Maui Sheep Milk, Hamilton, New Zealand), Steph Mann (Te Pūkenga | Otago Polytechnic, Dunedin, New Zealand), the many VetOra (North Island, New Zealand) technicians, and the students who assisted on farm. The cooperation of the study farmers is also acknowledged. Supplemental material for this article is available at https://doi.org/10.6084/m9.figshare.30524183. All animal manipulations in this study were approved by the Massey University Animal Ethics Committee (application AEC 22/25). The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: CMT = California Mastitis Test; ICC = intraclass correlation coefficient; OR = odds ratio; ROC AUC = area under the receiver-operating characteristic curve.

REFERENCES

Alba, D. F., G. da Rosa, D. Hanauer, T. F. Saldanha, C. F. Souza, M. D. Baldissera, D. da Silva Dos Santos, A. P. Piovezan, L. K. Girardini, and A. Schafer Da Silva. 2019. Subclinical mastitis in Lacaune sheep: Causative agents, impacts on milk production, milk quality, oxidative profiles and treatment efficacy of ceftiofur. Microb. Pathog. 137:103732. https://doi.org/10.1016/j.micpath.2019.103732.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, D. T. Galligan, M. L. Beiboer, and A. Brand. 1997. Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. J. Dairy Sci. 80:1592–1599. https://doi.org/10.3168/jds.S0022-0302(97)76089-2.

Bennett, S., T. Woods, W. M. Liyanage, and D. L. Smith. 1991. A simplified general method for cluster-sample surveys of health in developing countries. World Health Stat. Q. 44:98–106.

Berthelot, X., G. Lagriffoul, D. Concordet, F. Barillet, and D. Bergonier. 2006. Physiological and pathological thresholds of somatic cell counts in ewe milk. Small Rumin. Res. 62:27–31. https://doi.org/10.1016/j.smallrumres.2005.07.047.

Bianchi, L., C. Casoli, M. Pauselli, E. Budelli, A. Caroli, A. Bolla, and E. Duranti. 2004. Effect of somatic cell count and lactation stage on sheep milk quality. Ital. J. Anim. Sci. 3:147–156. https://doi.org/10.4081/ijas.2004.147.

Booth, J. G., and J. P. Hobert. 1998. Standard errors of prediction in generalized linear mixed models. J. Am. Stat. Assoc. 93:262–272. https://doi.org/10.1080/01621459.1998.10474107.

Chambers, G., R. Laven, A. Grinberg, A. Ridler, and N. Velathanthiri. 2024. An observational study of farmer-reported clinical mastitis in New Zealand dairy ewes. N. Z. Vet. J. 72:212–224. https://doi.org/10.1080/00480169.2024.2344566.

Chambers, G., K. E. Lawrence, A. Grinberg, N. Velathanthiri, A. Ridler, and R. Laven. 2026. Subclinical mastitis in New Zealand grazing dairy ewes 2: Relationships among somatic cell count, California Mastitis Test, and milk culture, and risk factors for elevated aerobic plate count. J. Dairy Sci. 109:705–721. https://doi.org/10.3168/jds.2025-27076.

Chambers, G., K. E. Lawrence, A. L. Ridler, and R. A. Laven. 2025. Teat and udder morphology and pathology of New Zealand dairy ewes. N. Z. Vet. J. 73:246–259. https://doi.org/10.1080/00480169.2025.2456240.

Cuccuru, C., M. Meloni, E. Sala, L. Scaccabarozzi, C. Locatelli, P. Moroni, and V. Bronzo. 2011. Effects of intramammary infections on somatic cell score and milk yield in Sarda sheep. N. Z. Vet. J. 59:128–131. https://doi.org/10.1080/00480169.2011.562862.

DeLong, E. R., D. M. DeLong, and D. L. Clarke-Pearson. 1988. Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. Biometrics 44:837–845. https://doi.org/10.2307/2531595.

Fragkou, I. A., C. M. Boscos, and G. C. Fthenakis. 2014. Diagnosis of clinical or subclinical mastitis in ewes. Small Rumin. Res. 118:86–92. https://doi.org/10.1016/j.smallrumres.2013.12.015.

Fthenakis, G. C. 1994. Prevalence and aetiology of subclinical mastitis in ewes of southern Greece. Small Rumin. Res. 13:293–300. https://doi.org/10.1016/0921-4488(94)90078-7.

Gonzalo, C., A. Ariznabarreta, J. A. Carriedo, and F. San Primitivo. 2002. Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. J. Dairy Sci. 85:1460–1467. https://doi.org/10.3168/jds.S0022-0302(02)74214-8.

Gonzalo, C., M. T. Juárez, M. C. García-Jimeno, and L. F. De La Fuente. 2019. Bulk tank somatic cell count and total bacterial count are affected by target practices and milking machine features in dairy sheep flocks in Castilla y León region, Spain. Small Rumin. Res. 178:22–29. https://doi.org/10.1016/j.smallrumres.2019.07.007.

Griffiths, K. J., A. L. Ridler, C. Compton, R. A. Corner-Thomas, and P. R. Kenyon. 2019. Investigating associations between lamb survival to weaning and dam udder and teat scores. N. Z. Vet. J. 67:163–171. https://doi.org/10.1080/00480169.2019.1596523.

Hartig, F. 2022. DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models. Accessed Nov. 1, 2024. https://cran.r-project.org/web/packages/DHARMa/index.html.

Jaeggi, J. J., S. Govindasamy-Lucey, Y. M. Berger, M. E. Johnson, B. C. McKusick, D. L. Thomas, and W. L. Wendorff. 2003. Hard ewe's milk cheese manufactured from milk of three different groups of somatic cell counts. J. Dairy Sci. 86:3082–3089. https://doi.org/10.3168/jds.S0022-0302(03)73908-3.

Kenyon, P. R., S. K. Maloney, and D. Blache. 2014. Review of sheep body condition score in relation to production characteristics. N. Z. J. Agric. Res. 57:38–64. https://doi.org/10.1080/00288233.2013.857698.

Knuth, R. M., W. C. Stewart, J. B. Taylor, C. J. Yeoman, B. Bisha, C. M. Page, C. M. Rowley, B. C. Lindsey, M. L. Van Emon, and T. W. Murphy. 2019. Subclinical mastitis in sheep: Etiology and association with milk somatic cell count and ewe productivity in three research flocks in the Western United States. Transl. Anim. Sci. 3(Supplement 1):1739–1743. https://doi.org/10.1093/tas/txz078.

Las Heras, A., L. Domínguez, and J. F. Fernández-Garayzábal. 1999. Prevalence and aetiology of subclinical mastitis in dairy ewes of the Madrid region. Small Rumin. Res. 32:21–29. https://doi.org/10.1016/S0921-4488(98)00152-7.

Leitner, G., M. Chaffer, A. Shamay, F. Shapiro, U. Merin, E. Ezra, A. Saran, and N. Silanikove. 2004. Changes in milk composition as affected by subclinical mastitis in sheep. J. Dairy Sci. 87:46–52. https://doi.org/10.3168/jds.S0022-0302(04)73140-9.

Lenth, R. V. 2024. Emmeans: Estimated marginal means, aka least squares means. Accessed Nov. 1, 2024. https://rvlenth.github.io/emmeans/.

Lysitsas, M., G. Botsoglou, D. Dimitriadis, S. Termatzidou, P. Kazana, G. Tsoumakas, C. N. Tsokana, E. Malissiova, V. Spyrou, C. Billinis, and G. Valiakos. 2024. Subclinical mastitis in Lacaune sheep: Etiologic agents, the effect on milk characteristics, and an evaluation of infrared thermography and the YOLO algorithm as a preprocessing tool for advanced analysis. Vet. Sci. 11:676. https://doi.org/10.3390/vetsci11120676.

Margatho, G., H. Quintas, V. Rodriguez-Estevez, and J. Simoes. 2020. Udder morphometry and its relationship with intramammary infections and somatic cell count in Serrana goats. Animals (Basel) 10:1534. https://doi.org/10.3390/ani10091534.

Mavrogianni, V. S., P. J. Cripps, and G. C. Fthenakis. 2007. Bacterial flora and risk of infection of the ovine teat duct and mammary gland throughout lactation. Prev. Vet. Med. 79:163–173. https://doi.org/10.1016/j.prevetmed.2006.11.014.

McCoard, S., D. Stevens, D. Selbie, L. Day, W. Young, A. E.-D. Bekhit, and L. Samuelsson. 2023. Supporting the growth of the dairy sheep industry in New Zealand—Industry update and review of a programme linking industry and science. N. Z. J. Agric. Res. 68:183–217. https://doi.org/10.1080/00288233.2023.2272594.

Michael, C. K., D. T. Lianou, K. Tsilipounidaki, Z. Florou, N. G. C. Vasileiou, V. S. Mavrogianni, E. Petinaki, and G. C. Fthenakis. 2023a. Longitudinal study of antibiotic resistance of staphylococci from cases of subclinical mastitis in sheep in Greece: Incidence and risk factors. Antibiotics (Basel) 12:1703. https://doi.org/10.3390/antibiotics12121703.

Michael, C. K., D. T. Lianou, N. G. C. Vasileiou, V. S. Mavrogianni, E. Petinaki, and G. C. Fthenakis. 2023b. Longitudinal study of subclinical mastitis in sheep in Greece: An investigation into incidence risk, associations with milk quality and risk factors of the infection. Animals (Basel) 13:3295. https://doi.org/10.3390/ani13203295.

Ministry for Primary Industries and Massey University. 2020. New Zealand sheep dairy survey. Economic Intelligence Unit, New Zealand Ministry for Primary Industries, and Massey University.

R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sánchez, A., A. Contreras, J. Jiménez, C. Luengo, J. C. Corrales, and C. Fernández. 2003. Effect of freezing goat milk samples on recovery of intramammary bacterial pathogens. Vet. Microbiol. 94:71–77. https://doi.org/10.1016/S0378-1135(03)00066-X.

Schalm, O. W., and D. O. Noorlander. 1957. Experiments and observations leading to development of the California mastitis test. J. Am. Vet. Med. Assoc. 130:199–204.

Smith, E. M., E. M. Monaghan, S. J. Huntley, and L. E. Green. 2011. Short communication: Preliminary investigation into the effect of freezing and a cryopreservant on the recovery of mastitis pathogens from ewe milk. J. Dairy Sci. 94:4850–4855. https://doi.org/10.3168/jds.2010-4076.

Stevenson, M. A., E. Sergeant, and S. M. Firestone. 2024. epiR: Tools for the analysis of epidemiological data. Accessed Nov. 1, 2024. https://cran.r-project.org/web/packages/epiR/index.html.

Tzanidakis, N., C. N. Brozos, N. Voutzourakis, A. Stefanakis, E. Malama, D. Zoller, A. Zdragkas, J. Hickford, S. Sotiraki, and E. Kiossis. 2021. Effect of abiotic and biotic factors on subclinical mastitis occurrence in low-input dairy sheep production systems. Small Rumin. Res. 198:106341. https://doi.org/10.1016/j.smallrumres.2021.106341.

Vasileiou, N. G. C., P. J. Cripps, K. S. Ioannidi, D. C. Chatzopoulos, D. A. Gougoulis, S. Sarrou, D. C. Orfanou, A. P. Politis, T. C. Gonzalez-Valerio, S. Argyros, V. S. Mavrogianni, E. Petinaki, and G. C. Fthenakis. 2018. Extensive countrywide field investigation of subclinical mastitis in sheep in Greece. J. Dairy Sci. 101:7297–7310. https://doi.org/10.3168/jds.2017-14075.

Vasileiou, N. G. C., A. Giannakopoulos, P. J. Cripps, K. S. Ioannidi, D. C. Chatzopoulos, D. A. Gougoulis, C. Billinis, V. S. Mavrogianni, E. Petinaki, and G. C. Fthenakis. 2019a. Study of potential environmental factors predisposing ewes to subclinical mastitis in Greece. Comp. Immunol. Microbiol. Infect. Dis. 62:40–45. https://doi.org/10.1016/j.cimid.2018.11.011.

Vasileiou, N. G. C., V. S. Mavrogianni, E. Petinaki, and G. C. Fthenakis. 2019b. Predisposing factors for bacterial mastitis in ewes. Reprod. Domest. Anim. 54:1424–1431. https://doi.org/10.1111/rda.13541.

Vouraki, S., A. I. Gelasakis, I. J. Rose, and G. Arsenos. 2018. Risk factors of teat-end hyperkeratosis and its association with udder health in dairy ewes. J. Dairy Res. 85:44–49. https://doi.org/10.1017/S0022029917000887.

Wilson, E. B. 1927. Probable inference, the law of succession, and statistical inference. J. Am. Stat. Assoc. 22:209–212. https://doi.org/10.1080/01621459.1927.10502953.

Wu, S., C. M. Crespi, and W. K. Wong. 2012. Comparison of methods for estimating the intraclass correlation coefficient for binary responses in cancer prevention cluster randomized trials. Contemp. Clin. Trials 33:869–880. https://doi.org/10.1016/j.cct.2012.05.004.

ORCIDS

Greg Chambers, https://orcid.org/0000-0001-7864-0057
Kevin Lawrence, https://orcid.org/0000-0002-2453-1485
Alex Grinberg, https://orcid.org/0000-0003-3692-9711
Niluka Velathanthiri, https://orcid.org/0009-0005-0341-5759
Anne Ridler, https://orcid.org/0000-0002-5210-0578
Richard Laven https://orcid.org/0000-0002-8938-8595