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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

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ABSTRACT

Our objectives were, in grazing dairy ewes, (1) to describe SCC, California Mastitis Test (CMT) score, and ewe-level milk aerobic plate count (APC), (2) to explore the relationship between CMT and SCC, (3) to identify risk factors for elevated APC, and (4) to find the optimal SCC threshold for diagnosis of IMI. Gland-level milk samples were collected from ~15 randomly selected ewes on each of 20 New Zealand dairy sheep farms at early, mid, and late lactation in a repeated cross-sectional study. Aerobic bacterial culture and CMT (measured on a scale of 0, trace, 1, 2, or 3) were performed at the gland level, and SCC and APC at the ewe level using composite milk samples. Milk samples were collected from 893 ewes, 870 of which had complete SCC and culture data. Geometric mean SCC was 169,039 (95% CI: 153,921–185,641) cells/mL, varying between farms and decreasing across visits. A CMT score ≥1 in one or both glands occurred in 21.2% of ewes. Mean $\log_{10}$ SCC increased linearly with CMT score, but the correlation between the ewe's highest gland-level CMT score and SCC was moderate (Kendall's tau = 0.47, 95% CI 0.43–0.52). Bacteria were isolated from 86 (9.9%) ewes, with the most common bacteria being NAS (7.0% of glands) and *Staphylococcus aureus* (0.6% of glands). A SCC threshold of ~400,000 cells/mL had the greatest Youden's index for diagnosing IMI using a single SCC measurement. The APC was below the limit of quantification (1×10^3 cfu/mL) in 78.0% of ewes, and $<100 \times 10^3$ cfu/mL in 96.9% of ewes, and varied between visits and farms. Using a mixed Bayesian ordinal regression model, elevated CMT score and SCC, positive milk culture, and subclinical mastitis, but not udder asymmetry, were confirmed as risk factors for elevated APC. These findings provide baseline milk quality data for New Zealand grazing dairy ewes, confirm that udder health should be considered when investigating elevated bulk milk APC, and can be used to help producers manage SCC, subclinical mastitis, and APC, as well as informing further research. Findings specific to New Zealand's emerging sheep dairy industry offer a benchmark for pastoral systems internationally and highlight the importance of udder health to bulk milk quality.

Key words: sheep, milk quality, mastitis, aerobic plate 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., 2019), 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.

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

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

Meeting stringent milk quality standards is essential for maintaining access to international markets. Somatic cell count and aerobic plate count (**APC**) are widely used indicators of bulk milk quality. New Zealand producers who sell milk for human consumption are legally required to perform routine bulk milk SCC and APC testing. Processors typically set thresholds for bulk milk SCC and APC, and producers may be penalized for exceeding these thresholds. Some producers also perform routine milk recording to collect ewe-level milk quantity and quality information, including SCC.

Individual ewe SCC has been shown to have an association with IMI (Ariznabarreta et al., 2002; Gonzalo et al., 2002) and reduced milk yield (Gonzalo et al., 1994, 2002; Sutera et al., 2018) in dairy ewes, but systematic analyses of individual SCC for grazing dairy ewes are lacking. The CMT is anecdotally used by many New Zealand producers as a practical screening tool, especially at the end of the colostrum period or in response to high bulk milk SCC. The CMT has been shown to have a positive correlation with SCC and to be predictive of IMI in dairy ewes (McDougall et al., 2001), but the published research is limited.

Anecdotally, high bulk milk APC (typically defined as counts exceeding 100×10^3 cfu/mL) have occurred frequently and been a challenge to resolve on several New Zealand dairy sheep farms. Producers report that high counts persist after ruling out plant hygiene and refrigeration problems, which are known causes of elevated bulk milk APC (Jayarao et al., 2004). Some have observed reductions in bulk milk APC following flock screening with the CMT and removal of high-CMT ewes from supply. This has led to the hypothesis that mastitis may be a contributor to high bulk milk APC on dairy sheep farms. However, this hypothesis has not been formally tested, and it is possible that changes in APC were coincidental. Although APC has been described at the bulk milk level for dairy sheep farms (de Garnica et al., 2013; Gonzalo et al., 2019; Lianou et al., 2021), ewe-level APC data could not be found. Describing ewe-level APC and identifying its risk factors may help to identify whether individual ewes are the principal source of high bulk milk APC in pastoral systems.

Milk culture is not routinely performed on commercial farms due to the labor, cost, and time required. Instead, SCC and CMT are commonly used as indicators of subclinical mastitis. Somatic cell count thresholds for IMI have been proposed (González-Rodríguez et al., 1995; Berthelot et al., 2006; Lafi, 2006; Riggio et al., 2013), but some variation exists in the thresholds and in the methods used to determine them, as well as in the study populations, and large-scale studies performed on grazing systems are lacking.

Our objectives were to systematically describe individual ewe SCC, CMT, APC, and milk bacteriology results; to determine the correlation between CMT and SCC; to identify risk factors for elevated ewe milk APC; and to set thresholds for diagnosing IMI, among grazing dairy ewes on multiple New Zealand farms. This fills a global gap by focusing on pasture-based systems outside of 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, it was estimated that at the start of the 2022–2023 milking season there were ~40 commercial farms. 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. Assuming a prevalence of 26% (Vasileiou et al., 2018), and an intraclass correlation coefficient 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% CI that has a precision (half the width of the confidence interval) of 5.9%. Enrolling 15 ewes per visit from 20 farms was calculated to have a precision of 6.8%. Further details on the sample size calculation are available in Chambers et al. (2026).

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, all farms were seasonal, and lambing occurred entirely in the spring, except for one 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, one-year-old ewes, or other ewes during bad weather).

Milk quality assessments 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 (3 farms) or prolonged adverse weather (1 farm).

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. On the first 3 farms, at the first visit, the 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 milking at the morning milking at all 3 visits. The ewes were returned to the milking flock after examination.

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 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) and summarized in Chambers et al. (2026).

All procedures were carried out in the milking parlor by trained technicians or the lead author, with ewes in a standing position. 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). 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 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 divided into 2 polypropylene vials 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 and APC 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).

Laboratory Procedures. Somatic cell count and APC were 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). The APC was estimated by incubating samples at 30°C for 72 h on milk plate count agar and counting the number of colonies to calculate the number of colony-forming units per milliliter of milk. Plates with no colony-forming units were reported as $<1 \times 10^3$ cfu/mL, and plates with $>3 \times 10^6$ cfu/mL were reported as $>3 \times 10^6$ cfu/mL. The aerobic culture procedure has been previously described (Chambers et al., 2024) and is summarized in Chambers et al. (2026).

Statistical Analysis

Results of aerobic culture, APC, 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). The 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.

We collected data at the gland level (CMT score and aerobic culture results) and ewe level (ewe demographic information, SCC, and APC). Aerobic culture and CMT results were collapsed to the ewe level as described subsequently.

SCC and California Mastitis Test. Being heavily skewed, SCC was reported as raw and $\log_{10}$ SCC and categorized into "normal" (<500 × 10^3 cells/mL), "intermediate" (between 500 × 10^3 and 1 × 10^6 cells/mL), or "high" (>1 × 10^6 cells/mL) according to the system of Fragkou et al. (2014). Confidence intervals for the geometric mean SCC were obtained by applying a Wald (normal) CI to the mean of $\log_{10}$ SCC and back-transforming ($10^{\wedge x}$), and the Wilson method for the categorized SCC (Wilson, 1927).

Differences in SCC between visits and between farms were tested by constructing Bayesian regression models with a Student *t*-distribution due to the non-normal distribution of $\log_{10}$ SCC using the R brms package (Bürkner, 2021). The association between visit and $\log_{10}$ SCC was tested by including visit as the only fixed effect alongside a random intercept for farm. The variance was allowed to vary between visits in the model due to heteroscedasticity. The association between farm and $\log_{10}$ SCC was tested by including farm as a fixed effect. The models were run with 4 chains of 2,000 iterations each, with a burn-in of 1,000 iterations and a thinning rate of 1. All models were checked for convergence and mixing by inspecting $\hat{R}$ values (which should be close to 1), effective sample sizes (the number of independent posterior samples), trace plots of Markov chains (checking for stable chains), and plots of posterior distributions and their pairwise relationships. Overfitting and influential observations (and therefore mis-specified models) were checked by computing Pareto *k*-values, with values >0.7 indicating potential issues. The statistical significance of visit and farm were determined by leave-one-out cross-validation (**LOO-CV**) in comparison to an analogous model without the fixed effect of interest. This is a method for comparing the predictive power of Bayesian regression models by sequentially leaving out one observation, fitting the model to the remaining data, and predicting the left-out observation. Models were compared on the expected log point-wise predictive density (**ELPD**) computed from LOO-CV, with a difference more than 2 times the SE of the difference taken as significant. The ELPD is a measure of how well a model predicts new data. It is computed as the sum of the log-likelihoods for each observation, averaged over the posterior distribution, with each observation left out in turn. Higher ELPD values indicate better predictive performance. Goodness of fit was also checked by plotting actual versus predicted distributions of APC.

Because CMT was measured at the gland level but SCC at the ewe level, CMT scores were collapsed to the ewe level by taking the maximum and median score of the 2 glands. The median score was calculated by converting the ordinal categories to integers (negative = 0; trace = 1; 1 = 2; 2 = 3; 3 = 4), taking the median of the 2 glands (rounded up to the nearest whole number), and converting back to the original scale. The relationship between CMT and SCC was appraised with boxplots of $\log_{10}$ SCC for each maximum and median CMT score and by computing summary statistics of SCC for each CMT score. Agreement between CMT and SCC was calculated with Kendall's τ-A. The Wilson method was also used to generate confidence intervals for the proportions of glands in each CMT score.

Aerobic Plate Count. The minimum limit of quantification for APC was 1 × 10^3 cfu/mL, below which results were reported as <1 × 10^3 cfu/mL, and the maximum limit of quantification was 3 × 10^6 cfu/mL, above which results were reported as 3 × 10^6 cfu/mL. The APC results of "quality control" (deviations from the protocol, n = 10) and "spreader" (colonies that spread across the plate and obscure other colonies, n = 1) were excluded from the analysis of APC. For descriptive purposes, median and interquartile range (**IQR**) were calculated for raw APC by converting values below the minimum limit of quantification to 500 cfu/mL and leaving results above the maximum limit of quantification as 3 × 10^6 cfu/mL. For all other analyses, APC was categorized into 4 groups because it was not a truly continuous variable due to its censored distribution: (1) <1,000; (2) 1,000–9,999; (3) 10,000–99,999; and (4) ≥100,000 cfu/mL. We also dichotomized APC into "low" (<100 × 10^3 cfu/mL) and "high" (≥100 × 10^3 cfu/mL) because 100 × 10^3 is a commonly used raw milk quality threshold. Confidence intervals for proportions in the categorized and dichotomized APC were calculated using the Wilson method. Differences between farms in the proportions of samples exceeding APC of 100 × 10^3 cfu/mL were tested with the Fisher exact test. Differences between visits were not explored until the risk factor stage of the analysis.

Bacterial Culture. Bacterial culture results were descriptively reported at the gland level in Chambers et al. (2026). Results were collapsed to the ewe level by categorizing ewes into having IMI due to (1) *Staphylococcus aureus* if either gland was positive for *S. aureus*; (2) NAS if *S. aureus* was not isolated but NAS was cultured from either gland; (3) "other"; (4) "contaminated" if either gland was contaminated and the other gland was culture-negative or also contaminated; (5) "mixed" if the 2 glands returned different results (from the set of NAS, *S. aureus*, and other) and neither gland was infected with *S. aureus* or contaminated; or (6) "no growth" if no bacteria were isolated from both glands. When one gland returned an unidentifiable isolate and the other was also unidentifiable or a no growth, the ewe was classified as "other." If one was unidentifiable and the other was positive and identifiable, the ewe was classified as "mixed." Ewes were regarded as having a positive bacterial culture if at least 1 gland had an identified bacterial isolate and neither gland was contaminated. Analysis was performed

on all ewes with culture results, but ewes with a contaminated result from either gland were excluded.

Subclinical Mastitis. Subclinical mastitis was defined at the ewe level as a bacteriologically positive milk sample (not contaminated) in 1 or both glands and having a CMT score ≥1 or SCC >500 × 10^3 cells/mL, or both (Fragkou et al., 2014). A bacteriologically positive milk sample with no increased CMT score (<1) or SCC <500 × 10^3 cells/mL was defined as "mammary carriage" (Vasileiou et al., 2018) and deemed not to have subclinical mastitis. The proportions of ewes in each combination of high and low SCC, CMT, and positive and negative culture were calculated.

Risk Factors for Elevated Aerobic Plate Count. Risk factor analysis for elevated categorized ewe milk APC was performed by constructing Bayesian ordinal regression models with a random intercept for farm with the brms package (Bürkner, 2021). Weakly informative priors (normally distributed with mean = 0 and SD = 2) were chosen for the random intercept and fixed effects coefficients. Candidate risk factors were maximum CMT score, median CMT score, dichotomized CMT score (0–1 or 2–3), dichotomized CMT score (0 to trace, or 1–3), $\log_{10}$ SCC, bacterial culture result (*S. aureus*, NAS, other, no growth), positive bacterial culture (any pathogen), subclinical mastitis, udder asymmetry, and visit. Each model contained a single explanatory variable and a random intercept for farm only, due to correlation between subclinical mastitis, SCC, CMT, and bacterial culture. Analysis was conducted on a complete case basis, as <5% of data were missing and the reasons for missingness were not related to the outcome (they were due to misplaced samples or recording errors causing samples to be discarded). The models were run with 4 chains of 2,000 iterations each, with a burn-in of 1,000 iterations and a thinning rate of 1. The same model diagnostic procedures were used as those for SCC. The statistical significance of each risk factor was determined by LOO-CV in comparison to a null model with only a random intercept for farm. The results were reported as odds ratios with 95% credible intervals (**CrI**). Population-average predicted probabilities of each APC category were calculated for each explanatory variable by drawing from the posterior distribution of the model for each level of a risk factor, or specific values of $\log_{10}$ SCC. Mean and 2.5% and 97.5% quantiles of the predictions were plotted as a function of the explanatory variable.

The assumption of proportional odds was tested by performing stratified analyses, in which separate binary logistic regression models employing different dichotomizations of APC categories were compared (i.e., <1,000 vs. 1,000–9,999, 10,000–99,999, and ≥100,000; <1,000 and 1,000–9,999 vs. 10,000–99,999 and ≥100,000; and <1,000, 1,000–9,999, and 10,000–99,999 vs. ≥ 100,000). The explanatory variable coefficients were compared to ensure they changed approximately monotonically across models. In addition, the Brant test was performed on analogous frequentist proportional odds models. Finally, analogous adjacent-category models were made, which do not assume proportional odds, and compared using the ELPD. If the adjacent-category model had a significantly better fit, the proportional odds assumption was rejected.

To assess prior sensitivity, we compared each model to an analogous model with default uninformative priors; that is, uniform distributions for fixed effects, and Student *t*-distributions (3 df, mean = 0, scale = 2.5) for intercepts and SD. If the posterior means and SD changed by at most 20% and the ELPD comparison was not significant, the priors were accepted.

SCC Thresholds for Predicting Intramammary Infection. The distribution of $\log_{10}$ SCC was summarized for culture-positive and negative ewes, and for ewes diagnosed with *S. aureus* or NAS IMI. To determine significant differences in $\log_{10}$ SCC between culture results, mixed linear regression models with a random intercept for farm were constructed, with one model for the presence of any IMI and a second model for culture result categorized by etiology. Pairwise contrasts were made for each culture result compared with the no-growth category. Contrasts for categorized culture results were adjusted for multiple comparisons with the Šidák method (Šidák, 1967).

Receiver operating characteristic (**ROC**) curves were constructed to assess the sensitivity and specificity of SCC for predicting IMI. Separate ROC curves were made for predicting any IMI, *S. aureus* IMI, and NAS IMI. The optimal $\log_{10}$ SCC threshold was determined by maximizing Youden's index, defined as the sum of sensitivity and specificity minus 1. The sensitivity, specificity, positive predictive value, negative predictive value, and Youden's index were calculated for SCC thresholds of 250 × 10^3, 500 × 10^3, 750 × 10^3, and 1 × 10^6 cells/mL.

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 or data analysis stages. Visits 1 through 3 were conducted respectively on August 24 to October 6, 2022; November 7 to December 22, 2022; and January 25 to March 16, 2023. Outside of the first 3 visits, more than 15 ewes were examined at 5 farm visits due to farmer selection error and having enough time to enroll more ewes. Only 12 ewes were examined at 1 farm visit due to farmer error in

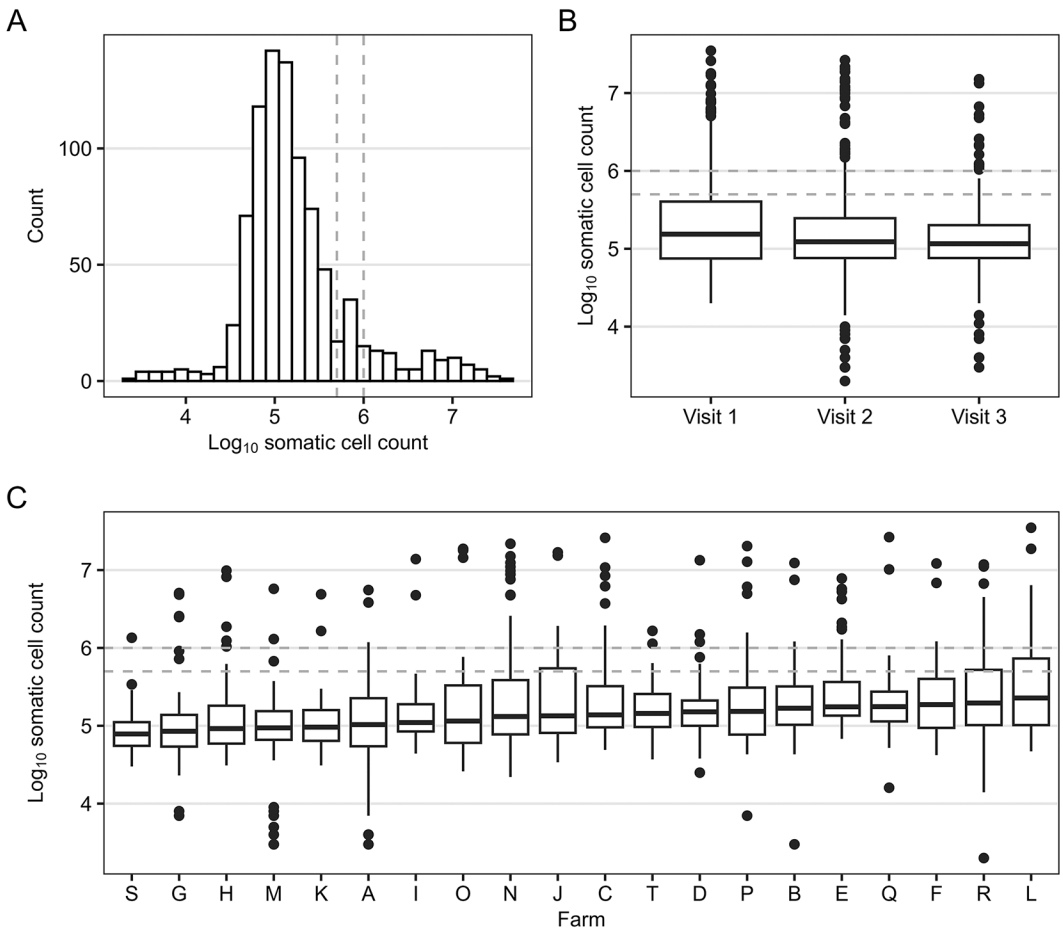

Figure 1. Distributions of $\log_{10}$ SCC (A) overall, (B) by visit, and (C) by farm (A–T; ordered on mean log SCC), in a study of milk quality of randomly selected ewes (n = 894) on 20 commercial dairy sheep farms in New Zealand. Boxes extend from the first to the third quartiles, with a horizontal line at the median. Upper whiskers extend from the third quartile to the highest value that is no more than 1.5 times the IQR from the third quartile. Lower whiskers extend from the first quartile to the lowest value that is no more than 1.5 times the IQR from the first quartile. Data beyond the end of the whiskers are deemed outliers and are plotted individually. Dashed lines indicate SCC of 500×10^3 and 1×10^6 cells/mL.

separating the ewes from the main flock. Complete demographic and examination data were available for 332 observations. The numbers of ewes examined on each farm at each visit are summarized in the supplemental material of Chambers et al. (2025), and the reasons for missing data are summarized in the supplemental material of the present article (Supplemental Table S1, see Notes). Farm and ewe information are summarized in Chambers et al. (2026).

SCC

Across all visits, SCC data were available for 890 ewes, with a median (IQR) 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. The mean log SCC was 5.2 (SD = 0.6), and the geometric mean SCC was 169,039 (95% CI: 153,921–185,641) cells/mL.

In total, 748, 53, and 89 samples had normal, intermediate, and high 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.

The distribution of log SCC is shown overall, and by visit and farm, in Figure 1. A decline in $\log_{10}$ SCC was confirmed between visits 1 and 2 and visits 1 and 3, with estimated differences (95% credible intervals) of 0.09 (0.01–0.17) and 0.14 (0.07–0.22) respectively. A decline between visits 2 and 3 could not be confirmed, with a difference of 0.05 (−0.01 to 0.11). Farm geometric mean $\log_{10}$ SCC ranged from 83,842 to 308,348 cells/mL, and a between-farm difference was confirmed by the model. The variance in $\log_{10}$ SCC was relatively large within farms compared with the variance between farms, as indicated by the large spread within farms and minimal differences in median values across farms (Figure 1).

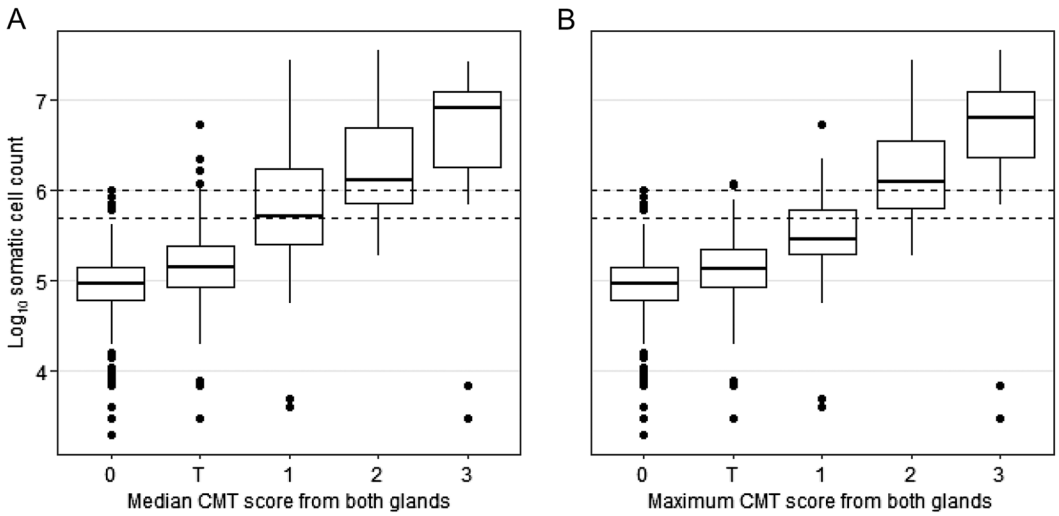

Figure 2. Distribution of $\log_{10}$ SCC for each California Mastitis Test (CMT) score when (A) ewes were assigned the median score of the 2 glands (rounded up to the nearest score), or (B) the maximum score, in a study of udder health of randomly selected ewes (n = 894) on 20 commercial dairy sheep farms in New Zealand. The CMT was scored using a scale of 0, trace (T), 1, 2, or 3. Boxes extend from the 25th to the 75th percentiles, with a line at the median. Whiskers extend to values no more than 1.5 times the IQR, and outliers are shown as individual dots. Dashed lines indicate SCC of 500×10^3 and 1×10^6 cells/mL.

California Mastitis Test

Data were available for 1,757 glands from 885 ewes. In all, 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 one gland of 189/893 (21.2%) ewes, with 108/189 (57.1%) being positive in a single gland only (i.e., the other gland had a score <1).

Agreement Between SCC and California Mastitis Test

Median $\log_{10}$ SCC increased approximately linearly across both median and maximum gland CMT scores (Figure 2). Medians and ranges of raw SCC, as well as the proportion of samples exceeding thresholds of 0.5×10^6 and 1×10^6 cells/mL, are shown for each CMT score in Table 1.

Median and maximum CMT score had almost identical correlations with $\log_{10}$ SCC, with Kendall's τ-B values of 0.46 (95% CI 0.41–0.5) and 0.47 (95% CI 0.43–0.52), respectively.

Aerobic Plate Count

The median and upper and lower quartiles of APC were all 500 cfu/mL (i.e., below limit of detection) across all visits, with a range of 500×10^3 to 3×10^6 cfu/mL. Overall, 682/874 (78%) milk samples had APC reported as <1,000 cfu/mL, and 847/874 (96.9%) <100,000 cfu/mL. The unadjusted proportions of samples in each APC category and above or below 100,000 cfu/mL are summarized overall and by visit in Table 2. We found differences between farms in the proportions of samples in each category ($P < 0.001$) and the proportion of samples exceeding 100,000 cfu/mL ($P < 0.001$), with no APC ≥100,000 cfu/mL recorded on 9/20 farms (Figure 3).

Risk Factors for Elevated Aerobic Plate Count

The distributions of APC risk factors are shown in Table 3, and the risk factor models are summarized in Table 4. Full model details are in the supplemental material (see Notes).

All models except the model for udder symmetry significantly improved the fit of the data compared with the null model. Based on ELPD, dichotomized CMT (categorized into scores 0–1 and 2–3) produced the best fit, followed by maximum CMT, subclinical mastitis, bacterial culture (positive or negative), bacterial class, median CMT, $\log_{10}$ SCC, and visit. Ewes with CMT scores 2 or 3 had 6.7 times (95% CrI = 4.36–10.22) higher odds of being in a higher APC category than ewes with CMT score of 0, trace, or 1. Though it did not fit the data as well as dichotomized CMT, subclinical mastitis was clearly associated with APC, given that ewes with subclinical mastitis had 10.77 times (95% CrI = 6.17–18.77) higher odds of being in a higher APC category than ewes without subclinical mastitis. Predicted probabilities of each

Table 1. Distribution of SCC for each ewe California Mastitis Test (CMT) score when ewes were assigned the median score of the 2 glands or the maximum score, in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand[1]

Method	N	Median (range) SCC	SCC >0.5 × 10^6 cells/mL	SCC >1 × 10^6 cells/mL
Median CMT				
0	440	94,000 (2,000–991,000)	5 (1.1%)	0 (0.0%)
T	265	139,000 (3,000–5,280,000)	23 (8.7%)	7 (2.6%)
1	95	508,000 (4,000–26,485,000)	48 (50.5%)	30 (31.6%)
2	45	1,299,000 (186,000–34,953,000)	40 (88.9%)	27 (60.0%)
3	27	8,192,000 (3,000–25,950,000)	24 (88.9%)	23 (85.2%)
Maximum CMT				
0	440	94,000 (2,000–991,000)	5 (1.1%)	0 (0.0%)
T	246	134,500 (3,000–1,166,000)	15 (6.1%)	4 (1.6%)
1	77	290,000 (4,000–5,280,000)	24 (31.2%)	5 (6.5%)
2	58	1,227,000 (186,000–26,485,000)	48 (82.8%)	31 (53.4%)
3	51	6,205,000 (3,000–34,953,000)	48 (94.1%)	47 (92.2%)

[1]CMT was scored on a scale of 0, trace (T), 1, 2, or 3. The CMT and SCC data were available for 870/893 ewes. The SCC data presented as n (%).

APC category are shown in Figure 4. The models met all the diagnostic criteria.

SCC Thresholds for Diagnosis of Intramammary Infection

Descriptive statistics for $\log_{10}$ SCC across bacteriological diagnoses are shown in Table 5. All kinds of IMI (i.e., any positive culture, NAS, or *S. aureus*) were shown to be associated with an increase in $\log_{10}$ SCC compared with no growth.

The diagnostic performance of SCC thresholds of 250,000, 500,000, 750,000, and 1,000,000 cells/mL, as well as the optimal SCC thresholds, for diagnosing IMI with any infection, *S. aureus*, and NAS, are shown in Table 6, and the ROC curves in Figure 5. Diagnosis of any IMI and NAS shared the same optimal SCC threshold of 406,443 cell/mL, whereas *S. aureus* had a higher optimal threshold at 799,834 cells/mL. The ROC areas under the curve (**AUC**) were 0.737 (95% CI 0.666–0.809), 0.89 (95% CI 0.783–0.998), and 0.748 (95% CI 0.666–0.829) for any IMI, *S. aureus*, and NAS, respectively.

DISCUSSION

We present the first systematic, large-scale description of SCC and CMT in grazing dairy sheep in New Zealand, a recently developed industry with distinctive features compared with established sheep dairying regions. We also introduce novel data on APC at the ewe level. This study confirms the suspicion held by many in the New Zealand dairy sheep industry that elevated APC can be associated with mastitis.

An anecdotal perception suggests that ewe SCC are generally much higher than cow SCCs, but this study did not support that, with a geometric mean of 169 × 10^3 cells/mL. We did find a substantial right tail for SCC among the ewes in our study. In fact, we found more ewes in the "high" (>1 × 10^6 cells/mL) category than the "intermediate" category (between 500 × 10^3 and 1 × 10^6 cells/mL)—10.0% and 6.0%, respectively—underscoring the value of monitoring ewe SCC by routine SCC measurement or screening with the CMT, or both.

The SCC detected in this study were low compared with those described by McDougall et al. (2001), who reported a geometric mean of 539 × 10^3 cells/mL for 258 New Zealand dairy ewes. Both studies examined foremilk collected in a similar manner, but the ewes in the McDougall et al. (2001) study were sampled at approximately 40 d postpartum, when they may have had higher SCC, as was seen in the present study with higher mean SCC at the first visit compared with later visits. In contrast, Ariznabarreta et al. (2002) found a mean $\log_{10}$ SCC of 4.86 and Gonzalo et al. (2002) found a mean $\log_{10}$ SCC of 4.98 cells/mL using similar methodology among bacteriologically negative dairy ewes in Spain. These SCC were approximately half the mean $\log_{10}$ of 5.16 observed in culture-negative ewes in the present study (Table 5). The reason for such a large difference between the studies is unclear, but it may reflect different management systems. The flocks in the study of Ariznabarreta et al. (2002) were not involved in any mastitis control programs, and those in the study of Gonzalo et al. (2002) participated in a recording scheme that included monthly milk testing. Such frequent testing is not standard on New Zealand dairy sheep farms.

Alternatively, freezing the milk samples between collection and culture may mean some of the culture-negative ewes in fact had IMI in our study. Smith et al. (2011) compared the recovery rate of bacteria in 50 ewe milk samples known to be infected after freezing for 4 or 8 wk. The proportion of samples that were bacteriologically positive declined by up to 50% with time across all pathogens, and the lower the colony-forming unit count

Table 2. Proportion of samples in each aerobic plate count (APC) category, overall and by visit, in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand

Item	Overall (n = 893)	Visit 1 (n = 286)	Visit 2 (n = 306)	Visit 3 (n = 301)
APC category (cfu/mL)				
<1,000	682/874 (78%)	213/285 (75%)	210/294 (71%)	259/295 (88%)
1,000–9,999	133/874 (15%)	54/285 (19%)	49/294 (17%)	30/295 (10%)
10,000–99,999	32/874 (3.7%)	10/285 (3.5%)	19/294 (6.5%)	3/295 (1.0%)
≥100,000	27/874 (3.1%)	8/285 (2.8%)	16/294 (5.4%)	3/295 (1.0%)
Missing	19	1	12	6
APC ≥100,000 cfu/mL	27/874 (3.1%)	8/285 (2.8%)	16/294 (5.4%)	3/295 (1.0%)
Missing	19	1	12	6

of the sample, the more its viability was altered by freezing. However, for culture-negative samples on d 0, freezing increased the isolation rate for *S. aureus*. Sánchez et al. (2003) demonstrated that freezing milk samples from goats infected with subclinical mastitis at −20°C or −80°C up to 730 d increased the colony-forming unit count of coagulase-negative staphylococci and reduced the colony-forming unit count of gram-negative bacilli with time when stored at −20°C, but not at −80°C. Schukken et al. (1989) demonstrated that freezing milk samples from cows with clinical or subclinical mastitis at −20°C for up to 16 wk reduced the number of samples that had cultures of *Escherichia coli* or *Actinomyces pyogenes*, increased the number of samples that had cultures of CNS, and had no effect on streptococci and *S. aureus*. These limited data suggest that freezing reduces the viability of some bacterial species in milk, especially in samples with low colony-forming unit counts, and deterioration appears to be greater for gram-negative pathogens. We cannot therefore rule out the possibility that some of the culture-negative samples in our study were actually positive, especially for gram-negative pathogens, thus causing the SCC of bacteriologically negative ewes to appear higher than they actually were.

Our SCC results may differ to those that would have been obtained through routine milk recording, which typically involves composite samples collected from the whole milk fraction during milking. We collected samples from the foremilk, not the whole fraction, and mostly 2 to 3 h after milking. The samples therefore reflect cisternal milk, not alveolar milk. However, McKusick et al. (2002) demonstrated lack of difference between cisternal and alveolar milk in SCC for samples collected from dairy ewes within 12 h of milking.

Substantial between-farm differences in $\log_{10}$ SCC were confirmed, with ~4-fold difference in mean SCC between the lowest and highest farms. The same phenomenon was seen by Gonzalo et al. (1994). However, the SCC variance within farm was relatively large compared with the variance between farms in our data set (Figure 1), indicating large differences between individual ewes managed on the same farm. This means that, at the industry level, there is scope to lower SCC on most farms by identifying high-SCC ewes, rather than just focusing on high-SCC farms. Gonzalo et al. (2002) also found farm to contribute little to the variance in SCC. In a statistical model containing flock, organism group, lactation stage, parity, type of birth, and interaction terms, flock explained only 0.6% of the variance.

A decline in SCC was observed across the season. The reason is unknown, but it may be a result of selective removal of high-SCC ewes from flocks, as we did not sample the same ewes at each visit, or a natural physiological decrease in SCC along the lactation. This aligns with the decline in subclinical mastitis prevalence reported in Chambers et al. (2026). A decline in SCC was observed among dairy ewes in the United States that were repeatedly sampled across lactation (Page et al., 2020). In contrast, Gonzalo et al. (1994) sampled the same ewes repeatedly and found an increase in SCC across time postpartum.

Using a cutoff of CMT score <1, 84.6% of ewes did not have an elevated SCC according to CMT. This aligns with the 84.0% of ewes that were categorized as "normal" (SCC $<500 \times 10^3$ cells/mL). Using the same scale, Fthenakis (1995) diagnosed 87% of ewes with CMT scores <1 and recommended a threshold of score 1 for diagnosing subclinical mastitis. Approximately half of the ewes with a CMT score ≥1 in at least 1 gland had scores ≥1 in both glands in the present study, meaning that the composite milk from such ewes will contribute relatively more somatic cells to the bulk milk, increasing their influence on reductions in bulk milk SCC when removed from supply.

We showed an approximately linear stepwise increase in mean $\log_{10}$ SCC and the proportion of ewes with SCC exceeding 0.5×10^6 or 1×10^6 cells/mL with increasing median or maximum CMT scores (Figure 2), and similar agreement for both CMT methods. However, while CMT corresponded well to median SCC, wide variation in SCC occurred at each CMT score, and hence the Kendall's τ scores were only moderately positive at 0.46 and 0.47 for median and maximum CMT, respectively. Several low-SCC outliers were visible (Figure 2) and may be due to

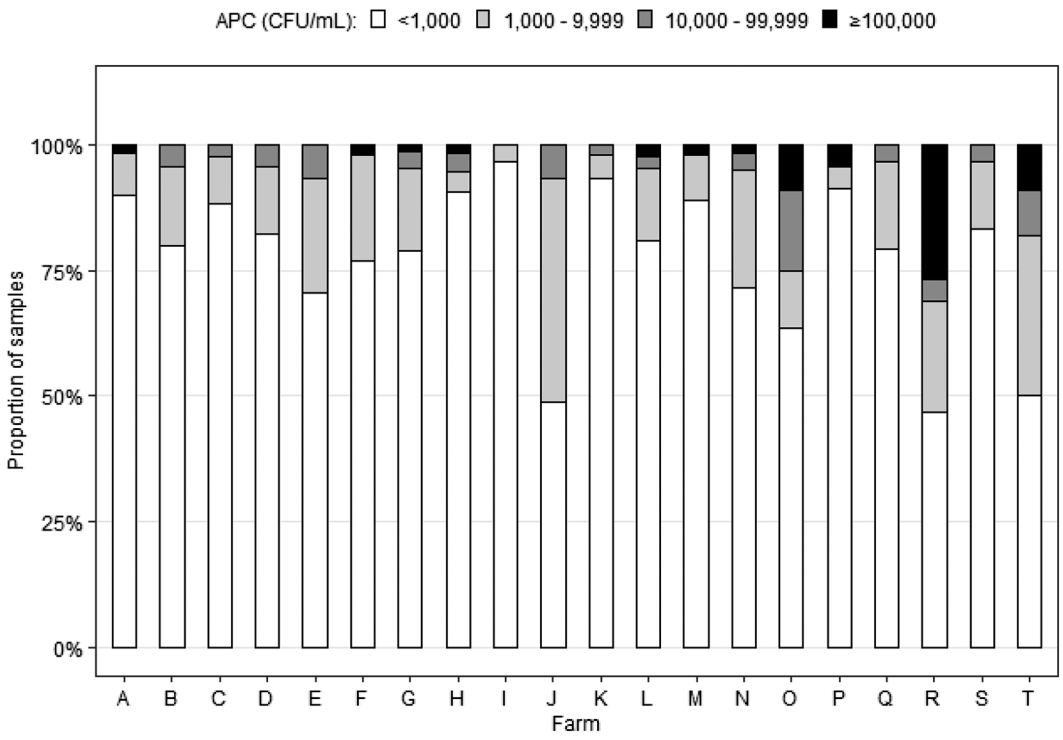

Figure 3. Distribution of aerobic plate count (APC) of ewe milk samples on each farm in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms (A–T) in New Zealand.

scoring or recording errors. This research supports CMT as a tool for identifying ewes with elevated SCC, but the wide variation in SCC at each CMT score means that it is not a perfect diagnostic tool and is subjective, again highlighting the value of routine SCC measurement.

Aerobic plate count is normally measured at the bulk milk level, so the present study was a departure from standard practice. Our motivation for measuring APC was to narrow down the source of elevated bulk milk APC. Most ewes had APC below the limit of quantification, which supports a generally robust sample collection and handling procedure. Only 3.1% of ewes had APC >100 × 10^3 cfu/mL, many of which were on one farm (Figure 3). Despite APC being generally low, we confirmed differences between visits and farms in the proportion of samples with APC >100 × 10^3 cfu/mL. The reasons for these differences are not clear, but may be due to differences in teat hygiene, presence of microbes in the milk, or sample handling. Steps were taken to mitigate between-farm differences in teat hygiene by cleaning teats in the same manner across all farms.

Several risk factors were confirmed for elevated APC at the ewe level. Overall, evidence suggests that IMI can contribute to APC, due to confirmed associations between ewe-level APC and CMT, SCC, culture, and subclinical mastitis. Udder asymmetry was not confirmed as a risk factor, in contrast to its strong association with subclinical mastitis (Chambers et al., 2026). This may be because udder asymmetry is a chronic change and, although it may be associated with SCC, ewes with asymmetric udders are not necessarily shedding large numbers of bacteria. *Staphylococcus aureus* had a weaker association with elevated APC than NAS and other or mixed IMI, suggesting that *S. aureus* IMI result in less bacterial shedding into the milk. The odds of elevated APC were lower at visit 3, which may reflect selective removal of ewes or the drier late summer weather.

Although significant associations were confirmed for these risk factors, they did not perfectly predict APC. Dichotomized CMT score was the best predictor of elevated APC. Ewes with CMT scores of 2 or 3 had 6.7 times (95% CI = 4.36–10.22) higher odds of being in a higher APC category than ewes with CMT scores of 0, trace, or 1. However, dichotomized CMT identified only 11/27 (41%) of ewes with APC ≥100 × 10^3 (Table 3). Furthermore, only 11/109 (10.1%) of ewes with high dichotomized CMT score had APC ≥100 × 10^3. Similarly, the predicted probabilities in Figure 4 do not account for the proportion of ewes in each risk factor category. For example, although the probability of elevated APC is clearly higher for ewes with dichotomized CMT scores of 2 to 3, only 109/874 (12.5% of ewes) had a CMT score of 2 to 3 (Table 3). Therefore, despite ewes with high

Table 3. Risk factor distributions overall and for each category of aerobic plate count (APC) in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand

Risk factor	Overall (n = 874)	<1,000 (n = 682)	1,000–9,999 (n = 133)	10,000–99,999 (n = 32)	≥100,000 (n = 27)
Median CMT[1]					
0	426 (50%)	354 (53%)	48 (36%)	14 (44%)	10 (37%)
T	262 (31%)	214 (32%)	37 (28%)	6 (19%)	5 (19%)
1	95 (11%)	64 (9.7%)	20 (15%)	4 (13%)	7 (26%)
2	45 (5.3%)	24 (3.6%)	14 (11%)	3 (9.4%)	4 (15%)
3	27 (3.2%)	7 (1.1%)	14 (11%)	5 (16%)	1 (3.7%)
Missing	19	19	0	0	0
Maximum CMT					
0	426 (50%)	354 (53%)	48 (36%)	14 (44%)	10 (37%)
T	244 (29%)	198 (30%)	35 (26%)	6 (19%)	5 (19%)
1	76 (8.9%)	63 (9.5%)	12 (9.0%)	0 (0%)	1 (3.7%)
2	58 (6.8%)	30 (4.5%)	15 (11%)	6 (19%)	7 (26%)
3	51 (6.0%)	18 (2.7%)	23 (17%)	6 (19%)	4 (15%)
Missing	19	19	0	0	0
Dichotomized CMT					
0–1	746 (87%)	615 (93%)	95 (71%)	20 (63%)	16 (59%)
2–3	109 (13%)	48 (7.2%)	38 (29%)	12 (38%)	11 (41%)
Missing	19	19	0	0	0
Bacterial culture class					
No growth	771 (90%)	629 (94%)	102 (78%)	22 (71%)	18 (67%)
NAS	60 (7.0%)	28 (4.2%)	23 (18%)	5 (16%)	4 (15%)
Other	16 (1.9%)	7 (1.0%)	4 (3.1%)	2 (6.5%)	3 (11%)
Mixed	3 (0.4%)	0 (0%)	0 (0%)	1 (3.2%)	2 (7.4%)
Staphylococcus aureus	5 (0.6%)	3 (0.4%)	1 (0.8%)	1 (3.2%)	0 (0%)
Missing	19	15	3	1	0
Bacterial culture (positive/negative)					
Negative	771 (90%)	629 (94%)	102 (78%)	22 (69%)	18 (67%)
Positive	85 (9.9%)	38 (5.7%)	28 (22%)	10 (31%)	9 (33%)
Missing	18	15	3	0	0
Subclinical mastitis					
Normal	784 (93%)	635 (97%)	105 (81%)	24 (75%)	20 (74%)
Subclinical mastitis	57 (6.8%)	17 (2.6%)	25 (19%)	8 (25%)	7 (26%)
Missing	33	30	3	0	0
Udder symmetry					
No	337 (39%)	253 (37%)	56 (42%)	13 (41%)	15 (56%)
Yes	535 (61%)	427 (63%)	77 (58%)	19 (59%)	12 (44%)
Missing	2	2	0	0	0
Visit					
1	285 (33%)	213 (31%)	54 (41%)	10 (31%)	8 (30%)
2	294 (34%)	210 (31%)	49 (37%)	19 (59%)	16 (59%)
3	295 (34%)	259 (38%)	30 (23%)	3 (9.4%)	3 (11%)

[1]CMT scored on a scale of 0, trace (T), 1, 2, or 3.

dichotomized CMT score having much higher odds of elevated APC, most ewes do not have a high CMT score, so a large number of high-APC ewes may be undiagnosed. Producers should therefore not expect to completely resolve an APC issue with CMT alone. As well as the diagnostic inaccuracy of CMT, random variation and plant hygiene or refrigeration issues may be the cause of high bulk milk APC. We propose that future research on APC should include repeatedly sampling ewes and bulk milk concurrently to quantify the proportion of bulk milk APC variation that is explained by ewe-level APC, and the temporal alignment.

Previous research has confirmed a positive correlation between SCC and total bacterial count (**TBC**) at the bulk milk level (de Garnica et al., 2013; Lianou et al., 2021). Additionally, Lianou et al. (2021) showed that isolation of *S. aureus* (but not NAS) from the bulk tank milk was associated with elevated TBC, and that the odds of elevated bulk milk TBC were significantly higher in the first month of the lactation period, which was thought to be related to housing. Our study also found an association between APC and SCC, but, in contrast to the results of Lianou et al. (2021), we found that APC at the first visit were lower than at the second visit. This difference is probably explained by the outdoor management of the vast majority of ewes in New Zealand. Further supporting a relationship between IMI and bulk milk APC, de Garnica et al. (2013) found that bulk milk TBC was lower in flocks that used dry ewe therapy than flocks that did not. This was confirmed by Gonzalo et al. (2019), who demonstrated associations between bulk milk TBC and 21 ewe, management, and plant factors.

Table 4. Odds ratios (95% CrI) of Bayesian mixed ordinal regression models of the associations between risk factors and aerobic plate count (categorized into <1,000, 1,000–9,999, 10,000–99,999, or ≥100,000 cfu/mL), ranked on significance, in a study of the milk quality of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand[1]

Risk factor model and fixed effect	Odds ratio (95% CrI)	ELPD[2]	Model significance[3]
Dichotomized CMT			
0–1	(Referent)	−536.6925	*
2–3	6.7 (4.36–10.22)		
Maximum CMT			
0	(Referent)	−537.3385	*
T	1.37 (0.88–2.19)		
1	0.87 (0.42–1.76)		
2	6.11 (3.24–10.94)		
3	8.87 (4.78–16.74)		
Subclinical mastitis			
Negative	(Referent)	−538.9855	*
Positive	10.77 (6.17–18.77)		
Bacterial culture (positive/negative)			
Negative	(Referent)	−542.5518	*
Positive	7.32 (4.46–11.77)		
Bacterial culture (class)			
No growth	(Referent)	−543.0292	*
Staphylococcus aureus	2.7 (0.42–17.04)		
NAS	5.65 (3.24–10.23)		
Other	10.62 (3.84–28.1)		
Mixed	34.92 (4.29–265.19)		
Median CMT			
0	(Referent)	−546.3494	*
T	1.3 (0.84–1.97)		
1	2.67 (1.59–4.71)		
2	5.02 (2.71–10.16)		
3	11.4 (5.5–24.66)		
Log_{10} SCC	2.37 (1.82–3.11)		*
Visit			
1	(Referent)	−555.9055	*
2	1.31 (0.89–1.97)		
3	0.35 (0.22–0.58)		
Udder symmetry			
Symmetrical	(Referent)	−572.7749	
Asymmetrical	1.32 (0.94–1.82)		

[1]CMT scored on a scale of 0, trace (T), 1, 2, or 3.

[2]Expected log point-wise predictive density (ELPD), computed from leave-one-out cross-validation.

[3]Models were compared on the difference in ELPD, with a difference more than 2 times the SE of the difference taken as significant (denoted with *).

Ewes with IMI due to any pathogen, NAS, or *S. aureus* had higher mean SCC than ewes with no IMI. Of the 3 pathogen groups, *S. aureus* had the highest SCC. However, as there were only 5 ewes with *S. aureus*, the data are also compatible with no significant difference between pathogen groups, so this should be interpreted with caution.

Somatic cell count showed good overall discriminatory ability based on ROC AUC, but application depends on the chosen threshold. Optimal SCC thresholds of ~400,000 cells/mL were identified for IMI due to any pathogen and NAS, and ~800,000 cells/mL for *S. aureus*. At those thresholds, the sensitivities for detecting IMI due to any growth and NAS were moderate at 0.64 and 0.67, respectively, and the specificities were 0.88 and 0.87, respectively. The sensitivity and specificity for *S. aureus* were higher, at 0.80 and 0.89, respectively. This suggests that a single SCC may be useful as a screening tool for detecting IMI, but confirmatory testing would be warranted. The AUC for all pathogens in our study was lower than the value of 0.90 calculated by McDougall et al. (2001), where the prevalence of IMI was also low (5%), but the etiology was not described. The difference may have been caused by the different timing of sampling, the etiology, or the effect of freezing samples before culture. In contrast, Riggio et al. (2013) calculated similar AUC for all (0.75), major (0.88), and minor pathogens (0.73).

Youden's index for *S. aureus* varied little between a threshold of 500,000 and the optimal threshold of 800,000 cells/mL, and the sensitivity remained the same. Therefore, SCC of 400,000 cell/mL would seem to be

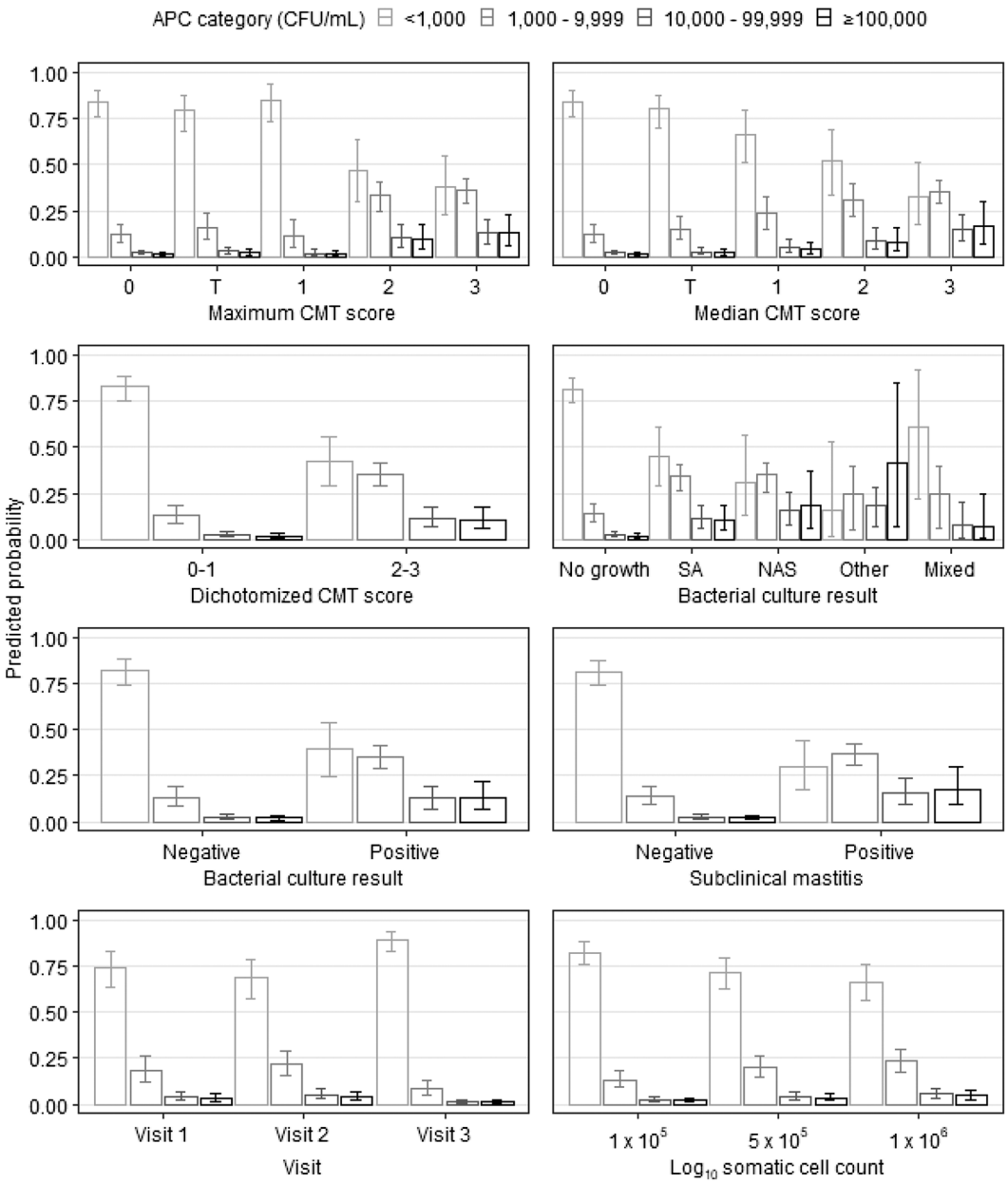

Figure 4. Predicted distributions of ewe-level aerobic plate count (APC) by California Mastitis Test (CMT) score, bacterial culture result, SCC, and visit, in a study of udder health among 893 ewes on 20 commercial New Zealand dairy sheep farms. Predictions were obtained from mixed Bayesian ordinal models with APC categorized as <1,000, 1,000–9,999, 10,000–99,999, and ≥100,000 cfu/mL. Error bars represent 95% Bayesian credible intervals.

a useful threshold for all pathogen groups based on our data. The mediocre sensitivities were mitigated by the low prevalence of infection, resulting in high negative predictive values, because a negative test result is more likely to mean a true negative when the prevalence is low. The specificities for all 3 pathogen groups were >0.8, but although specificity is less of a concern because positive ewes (ewes with SCC above the threshold) can be re-tested with culture to separate false positives, the positive predictive values were low due to the low prevalence. Farmers and advisors should therefore understand that, under New Zealand grazing conditions where the prevalence of IMI is relatively low, a single SCC <400,000 cells/mL is likely to truly indicate the ewe does not have an IMI, but more than 50% of ewes with a single SCC >400,000 cells/mL are in fact uninfected.

Several studies have reported SCC thresholds for diagnosing IMI in dairy sheep, but the prevalences and

Table 5. Mean (95% CI) $\log_{10}$ SCC for each milk culture result in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand; culture and SCC data were available for 870 ewes

Culture result	N (%)	Mean (95% CI)[1]	*P*-value[2]
No growth	784 (90.1)	5.16 (5.09–5.24)	
Any growth	86 (9.9)	5.88 (5.73–6.02)	<0.001
NAS	61 (7)	5.91 (5.74–6.07)	<0.001
Staphylococcus aureus	5 (0.6)	6.26 (5.73–6.79)	<0.001

[1]Predicted from a mixed linear regression model with a random intercept for farm.

[2]Test of the pairwise difference between each culture result and no growth.

Table 6. Optimal SCC thresholds for diagnosing IMI in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand[1]

IMI category and SCC threshold (cells/mL)	Se	Sp	PPV	NPV	YI
Any IMI					
250,000	0.651	0.772	0.238	0.953	0.423
406,443*	0.640	0.879	0.367	0.957	0.519
500,000	0.593	0.888	0.367	0.952	0.481
750,000	0.535	0.923	0.434	0.948	0.458
1,000,000	0.500	0.944	0.494	0.945	0.444
Non-*aureus* staphylococci					
250,000	0.689	0.761	0.179	0.970	0.450
406,443*	0.672	0.865	0.273	0.972	0.537
500,000	0.623	0.875	0.273	0.969	0.498
750,000	0.557	0.911	0.321	0.965	0.468
1,000,000	0.525	0.932	0.368	0.963	0.457
Staphylococcus aureus					
250,000	0.800	0.733	0.017	0.998	0.533
500,000	0.800	0.844	0.029	0.999	0.644
750,000	0.800	0.882	0.038	0.999	0.682
799,834*	0.800	0.891	0.041	0.999	0.691
1,000,000	0.600	0.903	0.034	0.997	0.503

[1]Thresholds marked with * are the best thresholds using Youden's index. Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; YI = Youden's index.

etiologies vary, and therefore comparisons between studies should be made carefully. Furthermore, we analyzed data at the ewe level, whereas some studies analyzed data at the gland level, which may result in higher SCC in the presence of IMI due to lack of dilution by the contralateral gland. However, we chose to use a ewe-level approach, because that is how routine flock recording is performed. Knuth et al. (2019) found that Youden's index was maximized at an SCC threshold of 1,375 × 10^3 cells/mL, but the IMI prevalence was 54% and dominated by *Bacillus* spp., whereas staphylococci dominated in the present study. In a study with an IMI prevalence of 36% that was dominated by staphylococci, the estimated optimal thresholds were 645,654 for any IMI and also for minor pathogens, and 2,137,962 for major pathogens (Riggio et al., 2013). However, as well as the higher prevalence of IMI, this study included economic considerations in selecting optimal thresholds instead of Youden's index, so their thresholds are not directly comparable. González-Rodríguez et al. (1995) identified a lower optimal threshold of 300,000 cells/mL in dairy ewes with a 44% prevalence of predominantly staphylococcal IMI. Their threshold was selected differently than the current study, being the threshold at which the false positives equaled the false negatives.

Our thresholds were based on a single SCC measurement, but using multiple SCC measurements may improve the accuracy of diagnosis. In the context of monthly milk recording, Berthelot et al. (2006) categorized ewes as healthy, doubtful or transiently infected, and infected or persistently infected. To be diagnosed as infected, ewes needed to have more than one SCC >1 × 10^6 cells/mL. This approach would require adaptation before applying it to other systems such as in New Zealand, where milk recording, when performed, may only occur 2 to 4 times in a lactation.

When viewed alongside international studies, our findings highlight both commonalities and differences between New Zealand grazing systems and established European and US systems. This study offers an insight into mastitis and milk quality among a new study population: grazing dairy ewes on New Zealand farms. In contrast to previous international research, these ewes typically spent most, if not all, of their time on pasture on farms that had been in operation for <10 yr. 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. Some of our findings align with those from more established dairy sheep regions, but others differ. The mean SCC of the subset of New Zealand ewes without IMI was between the values reported in Spain (Ariznabarreta et al., 2002; Gonzalo et al., 2002) and those reported from Sardinia (Cuccuru et al., 2011). We found a proportion of ewes with CMT score <1 similar to that reported by Fthenakis (1995). The SCC threshold that optimized diagnostic accuracy for IMI was lower than that of Knuth et al. (2019) in the United States and Riggio et al. (2013) in Italy, but higher than that of González-Rodríguez et al. (1995) in Spain, though the optimal threshold is affected by the prevalence of infection and the method used to select it. Importantly, our ewe-level APC results extend beyond bulk tank studies from Europe (de Garnica et al., 2013; Gonzalo et al., 2019; Lianou et al., 2021), indicating for the first time that subclinical mastitis can directly contribute to elevated APC under grazing conditions. These comparisons emphasize that, although the New Zealand industry is small and emerging, its baseline udder health indicators are broadly in line with those reported internationally, with the distinctive contribution of ewe-level APC representing a novel addition to the literature.

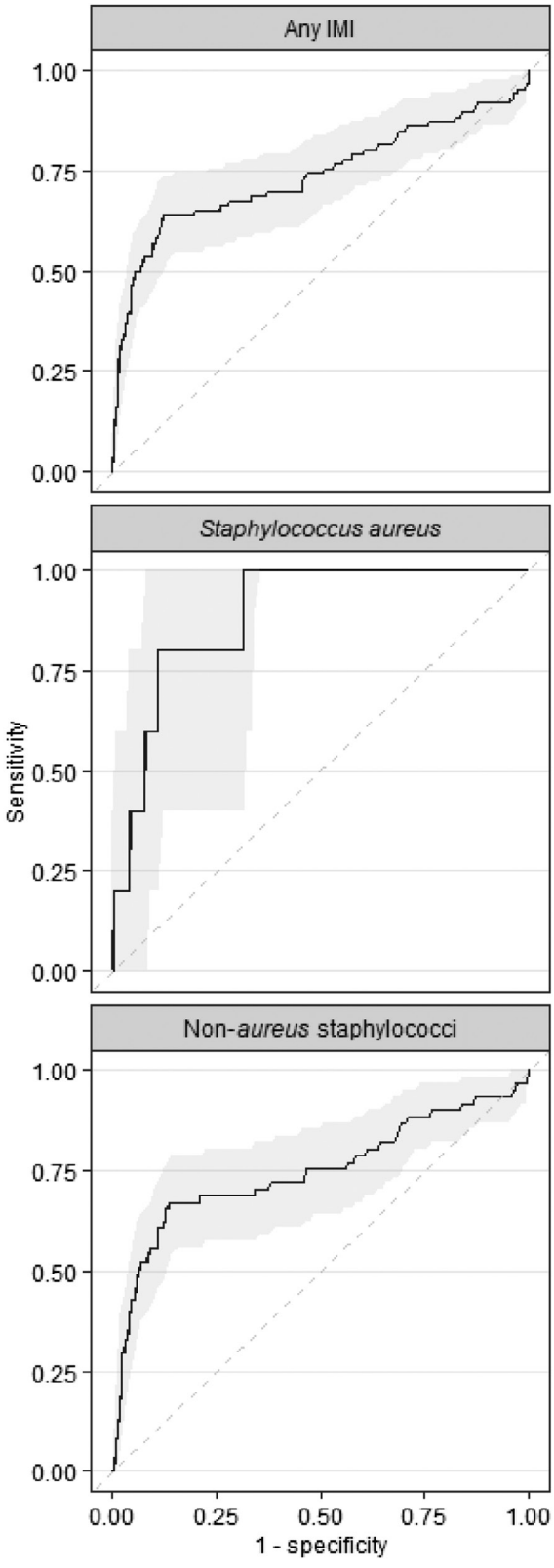

Figure 5. Receiver operator characteristic curves of the performance of SCC at diagnosing IMI in a study of udder health of randomly selected ewes (n = 893) on 20 commercial dairy sheep farms in New Zealand. Shaded gray areas represent 95% CI.

Our data supplement the international literature on milk quality and mastitis among dairy ewes and will be useful for farmers and industry partners to compare their own flocks to the results in this data set, identify areas for improvement, and inform and compare future studies. The present study could be extended by running a longitudinal study of ewe and bulk milk APC, validating the diagnosis of IMI by using multiple SCC measurements in systems similar to those in New Zealand, and including economic considerations in SCC threshold selection.

CONCLUSIONS

This study has established baseline information on the distribution of SCC, CMT, and APC in grazing dairy ewes. We found a decline in SCC over the lactation, and most variation was within rather than between farms. Mean $\log_{10}$ SCC increased linearly with CMT score, but the correlation between CMT score and SCC was only moderate. Elevated CMT score and SCC, positive milk culture, and subclinical mastitis, but not udder asymmetry, were confirmed as risk factors for elevated APC. An SCC threshold of 400,000 cells/mL had the greatest accuracy at diagnosing IMI using a single SCC measurement. These findings provide a baseline for milk quality and udder data in grazing dairy ewes and can be used to plan further research on SCC, CMT, APC, bacteriology, and subclinical mastitis. Although specific to New Zealand's emerging sheep dairying industry, these findings provide a useful benchmark for other pastoral-based production systems internationally, highlighting the need to consider udder health when addressing bulk milk quality issues.

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Nonstandard abbreviations used: APC = aerobic plate count; AUC = area under the curve; CMT = California Mastitis Test; CrI = credible intervals; ELPD = expected log point-wise predictive density; IQR = interquartile range; LOO-CV = leave-one-out cross-validation; NPV = negative predictive value; PPV = positive predictive value; ROC = receiver operating characteristic; Se = sensitivity; Sp = specificity; T = CMT score "trace"; TBC = total bacterial count; YI = Youden's index.

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